National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service

Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

NL-339 (r. 88/04) c

Canada

THE UNIVERSITY OF ALBERTA

NEUROTRANSMITTER ALTERATIONS IN PERITUMOR BRAIN

by

JACQUELINE BAYENS-SIMMONDS

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
EXPERIMENTAL SURGERY
DEPARTMENT OF SURGERY

EDMONTON, ALBERTA
FALL, 1988

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-52778-1

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: JACQUELINE BAYENS-SIMMONDS

TITLE OF THESIS: NEUROTRANSMITTER ALTERATIONS IN PERITUMOR BRAIN

DEGREE: Ph.D.

YEAR THIS DEGREE GRANTED: 1988

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

Jackie Bayens-Simmonds
6316 Fulton Drive
Edmonton, Alberta
T6A 3V3

Date: October 12 1988

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "NEUROTRANSMITTER ALTERATIONS IN PERITUMOR BRAIN", submitted by JACQUELINE BAYENS-SIMMONDS in partial fulfilment of the requirements for the degree of Doctor of Philosophy in EXPERIMENTAL SURGERY.

(Supervisor)

Glen B Baker
(Co-Supervisor)

Kenneth C Petruk

R G Coutts

(External Examiner)

Date: 3 October 1988

To my daughter Bridget and my husband Terry

ABSTRACT

After treatment with dexamethasone (DEX), neurologic improvement occurs in patients with brain tumors, preceding a reduction in brain edema; this may be due to an effect on neurotransmitters. To explore this possibility further, levels of noradrenaline (NA), dopamine (DA), 5-hydroxytryptamine (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole-3-acetic acid (5-HIAA), γ-aminobutyric acid (GABA) and tissue water content were measured in tumor tissue and peritumor (parietal), temporal, and frontal grey and white matter to determine if DEX treatment affected neurotransmitter levels in peritumor brain tissue.

A suspension of 10^6 9L glioma cells or medium alone was xenotransplanted into the parietal white matter of adult cats. The brains were frozen in vivo on day 15 or when neurologic signs were evident. Concentrations of NA and DA and its metabolites HVA and DOPAC were decreased in peritumor and temporal grey matter; all monoamine levels were unchanged in frontal grey matter. Cats with neurologic signs had the lowest concentrations of monoamines and their metabolites in their parietal and temporal grey matter, and increased GABA levels in their tumor, peritumor and frontal grey matter. Water content was increased in peritumor white matter but normal in grey matter.

Tumor-bearing cats without neurologic signs and control cats were treated or not treated with DEX (0.25 mg/kg i.v. and 0.25

mg/kg i.m.), with the i.m. dose repeated once (DEX schedule 1) or 3 times (schedule 2) at 6-h intervals. In peritumor grey matter, levels of NA, DA, and DOPAC were decreased; DEX 2 but not DEX 1 increased DA and DOPAC levels but neither schedule reduced white-matter edema. Tumor-bearing cats with neurologic signs were treated or not treated with DEX 1. Four of 5 treated animals improved neurologically. Treatment shifted regional monoamine and GABA levels towards control levels but did not reduce brain edema.

The findings indicate that DEX'S acute beneficial effect in patients with brain tumor may result from elevation of the depleted monoamine and metabolite levels and reduction of the elevated GABA levels.

KEY WORDS

brain neoplasm, 9L glioma, monoamines, γ-aminobutyric acid, dexamethasone

ACKNOWLEDGEMENTS

I am deeply indebted to my supervisors, Dr. Donald Boisvert and Dr. Glen Baker, for the opportunity to work in their laboratories. Their guidance and support are gratefully acknowledged.

I would like to thank Dr. E. Johnson for his interpretation of the histology and Dr. A. Greenshaw for many discussions on statistical analysis. I am appreciative of the time given by the members of my examining committee to read this thesis and for their valuable suggestions.

I am especially indebted to the following people:

- to Ms. V. Kervin, Ms. A. Elzanaty, Ms. H. Stelte and Ms. U. Mathews for expert editorial assistance.
- to the graduate students and staff of the Division of Neurosurgery, especially Dr. M. Castro, Dr. J. Lazareff and Mr. C. Schreiber, for their assistance in the surgical aspects of this study.
- to the graduate students and staff of the Neurochemical Research Unit, especially Dr. A. Nazarali, Dr. T.S. Rao, Dr. J. Yeung, and Mr. J. Wong, for their advice on the analysis of the tissue samples.
- to Dr. D. Neil and the staff of Health Sciences Laboratory Animal Services, especially Mr. G. Hawkins, Ms. L. Felgate, and Ms. M. Parker, for excellent care of the experimental animals.
- to Ms. V. Jeffry and Ms. C. Johnson for preparing tumor-cell cultures.
- to Mr. T. Turner for photography of histologic slides.

This study was supported by a postdoctoral fellowship from the Alberta Heritage Savings Trust Fund Applied Research - Cancer, and a University of Alberta research assistantship.

Finally, and most of all, I would like to thank my husband Terry for his support and patience during this long endeavour.

TABLE OF CONTENTS

CHAPTER ONE REVIEW OF THE LITERATURE

Page

1.1 **Introduction** 1

1.2 **Brain Tumor Models** 1
- 1.2.1 Virally induced brain tumor models 3
- 1.2.2 Chemically induced brain tumor models 5
- 1.2.3 Transplanted brain tumor models 6
- 1.2.4 Summary 7

1.3 **Effects of Brain Tumor on Brain Function** 9
- 1.3.1 Definition 9
- 1.3.2 Mass effect and intracranial pressure 9
- 1.3.3 Brain edema 10
- 1.3.4 Clinical neurologic status 12
- 1.3.5 Neurophysiology 14
- 1.3.6 Cerebral blood flow and oxygen utilization 15
- 1.3.7 Cerebral energy utilization 17
- 1.3.8 Arachidonic acid metabolism 19
- 1.3.9 GABA metabolism 21
- 1.3.10 Neurotransmitter amine metabolism 24
- 1.3.11 Summary 32

1.4 **Dexamethasone: Structure and Properties** 33

Page

1.5 Effects of Dexamethasone on Function of Normal Brain 36
1.5.1 Intracranial pressure 36
1.5.2 Clinical neurologic status 37
1.5.3 Brain water homeostasis 38
1.5.4 Neurophysiology 39
1.5.5 Cerebral blood flow and oxygen utilization 40
1.5.6 Cerebral energy utilization 40
1.5.7 Arachidonic acid metabolism 41
1.5.8 GABA metabolism 41
1.5.9 Neurotransmitter amine metabolism 42
1.5.10 Summary 44

1.6 Effects of Dexamethasone on Function of Tumor-Bearing Brain 45
1.6.1 Mass effect and intracranial pressure 45
1.6.2 Clinical neurologic status 47
1.6.3 Brain edema 47
1.6.4 Neurophysiology 49
1.6.5 Cerebral blood flow and oxygen utilization 50
1.6.6 Cerebral energy utilization 52
1.6.7 Arachidonic acid metabolism 53
1.6.8 GABA metabolism 54
1.6.9 Neurotransmitter amine metabolism 54
1.6.10 Summary 54

1.7 General Summary 56

CHAPTER TWO MATERIALS AND METHODS

Page

2.1 Experimental Animals 58

2.2 Brain Tumor Model 58
- 2.2.1 Culture of 9L-glioma cells 58
- 2.2.2 Preparation 59
- 2.2.3 Anesthesia 59
- 2.2.4 Surgery 59
- 2.2.5 Post-operative care 60

2.3 Administration of Dexamethasone 62
- 2.3.1 Schedule 1 (DEX 1) 62
- 2.3.2 Schedule 2 (DEX2) 62

2.4 Clinical Examination 62
- 2.4.1 Neurologic assessment 62
- 2.4.2 Measurement of intracranial pressure 62

2.5 Brain Tissue 65
- 2.5.1 Freezing in situ 65
- 2.5.2 Regional dissection of brain tissue 66

Page

2.6 **Equipment** 66

2.6.1 Gas chromatograph 66

2.6.2 High-pressure liquid chromatograph 70

2.6.3 Liquid-scintillation spectrometer 70

2.6.4 Glassware 71

2.6.5 Block heaters 72

2.6.6 Balances 72

2.6.7 Tissue homogenizers 72

2.6.8 Centrifuges 73

2.6.9 Shaker/mixer 73

2.7 **Chemicals** 74

2.8 **Tissue Measurements** 74

2.8.1 Measurement of water content 74

2.8.2 Analysis of regional brain levels of neurotransmitter amines and metabolites 77

2.8.3 Analysis of regional brain levels of GABA 77

2.8.4 Monoamine oxidase assay 81

2.9 **Pathology** 84

2.10 **Data Analysis** 84

CHAPTER THREE EXPERIMENTAL STUDIES

Page

3 The Problem 87

3.1 Study A 88
Characterization of the Feline Brain Tumor Model
    3.1.1 Hypothesis 88
    3.1.2 Objectives 88
    3.1.3 Design 88

3.2 Study B 90
Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites, and GABA in Untreated Tumor-Bearing Brain
    3.2.1 Hypothesis 90
    3.2.2 Objectives 91
    3.2.3 Design 91

Page

3.3 Study C 93
Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites and GABA, and Water Content in Tumor-bearing Brain of Cats without Abnormal Neurologic Signs: Effects of Two Dosage Schedules of Dexamethasone
3.3.1 Hypothesis 93
3.3.2 Objectives 94
3.3.3 Design 95

3.4 Study D 96
Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites and GABA, and Water Content in Tumor-Bearing Brain of Cats with Abnormal Neurologic Signs: the Effect of DEX 1 Dosage Schedule
3.4.1 Hypothesis 96
3.4.2 Objectives 96
3.4.3 Design 97

CHAPTER FOUR RESULTS

Page

4.1 **Study A** 100

Characteristics of the Feline Brain Tumor Model

4.1.1 Success rate of implantation and incidence of neurologic signs 100

4.1.2 Gross pathology 103

4.1.3 Microscopic pathology 103

4.1.4 Water content 110

4.1.5 Conclusions 110

4.2 **Study B** 111

Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites, and GABA in Untreated Tumor-Bearing Brain

4.2.1 Neurologic status 111

4.2.2 Intracranial pressure 112

4.2.3 Regional levels of neurotransmitter amines and their metabolites 112

4.2.4 Regional GABA levels 119

4.2.5 Conclusions 121

Page

4.3 **Study C** 122
Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites and GABA, and Water Content in Tumor-bearing Brain of Cats without Abnormal Neurologic Signs: Effects of Two Dosage Schedules of Dexamethasone
  4.3.1 Neurologic status 122
  4.3.2 Intracranial pressure 123
  4.3.3 Regional levels of neurotransmitter amines and their metabolites 123
  4.3.4 Regional GABA levels 139
  4.3.5 Regional water content levels 150
  4.3.6 Conclusions 151

4.4 **Study D** 153
Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites and GABA, and Water Content in Tumor-bearing Brain of Cats with Abnormal Neurologic Signs: The Effect of DEX 1 Dosage Schedule
  4.4.1 Neurological status 153
  4.4.2 Intracranial pressure 153
  4.4.3 Regional levels of neurotransmitter amines and their metabolites 155
  4.4.4 Monoamine oxidase inhibition 158
  4.4.5 Regional GABA levels 158

Page

4.4.6 Regional water content levels 160

4.4.7 Conclusions 162

CHAPTER FIVE DISCUSSION

5.1 **Study A** 163

Characteristics of the Feline Brain Tumor Model

5.2 **Study B** 167

Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites, and GABA in Untreated Tumor-bearing Brain

5.3 **Effect of Dexamethasone on Tumor-Bearing Brain** 177

5.3.1 Study C 177

Neurologic status, levels of neurotransmitter amines, their metabolites and GABA, and water content in tumor-bearing brain of cats without abnormal neurologic signs: the effect of two dosage schedules of dexamethasone

Page

5.3.2 Study D 179
Neurologic status, levels of neurotransmitter amines, their metabolites and GABA, and water content in tumor-bearing brain of cats with abnormal neurologic signs: the effect of DEX 1 dosage schedule
5.3.4 Summary 180

CHAPTER SIX 185
CONCLUSIONS AND RECOMMENDATIONS

REFERENCES 189

LIST OF TABLES

Page

Table 1. Description of neurologic scoring system 64

Table 2. List of chemicals used 75

Table 3. Tumor characteristics in cats without abnormal neurologic signs 101

Table 4. Tumor characteristics in cats with abnormal neurologic signs 102

Table 5. Neurotransmitter amine and metabolite levels in 9L tumor tissue and 9L glioma cells 113

Table 6. Neurotransmitter amine and metabolite levels in parietal grey matter of control and tumor-bearing cats 115

Table 7. Neurotransmitter amine and metabolite levels in temporal grey matter of control and tumor-bearing cats 116

Table 8. Neurotransmitter amine and metabolite levels in frontal grey matter of control and tumor-bearing cats 118

Page

Table 9. Levels of GABA in regional brain areas of control and tumor-bearing cats 120

Table 10. Summary of tumor and dexamethasone effects on NA levels in regional brain areas of control and tumor-bearing cats 140

Table 11. Summary of tumor and dexamethasone effects on DA levels in regional brain areas of control and tumor-bearing cats 141

Table 12. Summary of tumor and dexamethasone effects on DOPAC levels in regional brain areas of control and tumor-bearing cats 142

Table 13. Summary of tumor and dexamethasone effects on HVA levels in regional brain areas of control and tumor-bearing cats 143

Table 14. Summary of tumor and dexamethasone effects on 5-HT levels in regional brain areas of control and tumor-bearing cats 144

Table 15. Summary of tumor and dexamethasone effects on 5-HIAA levels in regional brain areas of control and tumor-bearing cats 145

Page

Table 16. Changes in neurologic scores of cats with neurologic signs of increased ICP after initial treatment with DEX 1 154

Table 17. Neurotransmitter amine and metabolite levels in parietal grey matter of cats with abnormal neurologic signs treated with DEX 1 156

Table 18. Neurotransmitter amine and metabolite levels in temporal grey matter of cats with abnormal neurologic signs treated with DEX 1 157

Table 19. Neurotransmitter amine and metabolite levels in frontal grey matter of cats with abnormal neurologic signs treated with DEX 1 159

Table 20. Levels of GABA in regional brain areas of cats with abnormal neurologic signs not treated or treated with DEX 1 161

LIST OF FIGURES

		Page
Figure 1.	Arachidonic acid metabolism	20
Figure 2.	Synthesis and metabolism of catecholamines	26
Figure 3.	Synthesis and metabolism of 5-HT	28
Figure 4.	Structures of cortisol, dexamethasone, and methylprednisolone	34
Figure 5.	Site of tumor-cell implantation	61
Figure 6.	Regional brain sampling areas in parietal brain, coronal view	67
Figure 7.	Regional brain sampling areas in frontal brain, coronal view	68
Figure 8.	Regional brain sampling areas, lateral view	69
Figure 9.	Typical HPLC run of authentic standards of NA, DA, 5-HT, DOPAC, HVA, and 5-HIAA	78
Figure 10.	Typical HPLC run on an extract of cat brain cortex	79

Page

Figure 11. Typical GC run of an authentic standard of GABA 82

Figure 12. Typical GC run on an extract of cat brain cortex 83

Figure 13. Flow chart illustrating number and subgroups of cats in each study 98

Figure 14. Coronal view of cat brain containing a 9L glioma tumor 104

Figure 15. Invasion of tumor along perivascular sheaths into surrounding brain. (magnification = 250 X) 109

Figure 16. Neurotransmitter amine and metabolite levels in tumors of cats not treated or treated with two dosage schedules of DEX 124

Figure 17. Levels of NA and DA in the parietal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 126

Page

Figure 18. Levels of DOPAC and HVA in the parietal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 128

Figure 19. Levels of 5-HT and 5-HIAA in the parietal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 130

Figure 20. Levels of NA and DA in the temporal grey matter of control and tumor-bearing cats treatedwith two dosage schedules of DEX 131

Figure 21. Levels of DOPAC and HVA in the temporal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 132

Figure 22. Levels of 5-HT and 5-HIAA in the temporal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 134

Figure 23. Levels of NA and DA in frontal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 135

Page

Figure 24. Levels of DOPAC and HVA in frontal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 136

Figure 25. Levels of 5-HT and 5-HIAA in frontal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 138

Figure 26. Levels of GABA in parietal, temporal, and frontal grey matter of control and tumor-bearing cats treated with two dosage schedules of DEX 147

Figure 27. Levels of GABA in parietal and frontal white matter of control and tumor-bearing cats treated with two dosage schedules of DEX 149

LIST OF ABBREVIATIONS

adrenocorticotropic hormone	ACTH
adenosine diphosphate	ADP
adenosine monophosphate	AMP
analysis of variance	ANOVA
avian sarcoma virus	ASV
adenosine triphosphate	ATP
blood-brain barrier	BBB
twice daily	b.i.d.
cyclic adenosine monophosphate	cAMP
cerebral blood flow	CBF
cerebral glucose utilization	CGU
catechol-O-methyltransferase	COMT
central nervous sytem	CNS
creatinine phosphate	CrP
computerized axial tomography	CT
dopamine	DA
dexamethasone	DEX
dimethylsulfoxide	DMSO
deoxyribonucleic acid	DNA
3,4-dihydroxymandelic acid	DOMA
3,4-dihydroxyphenylalanine	DOPA
3,4-dihydroxyphenylacetic acid	DOPAC
3,4-dihydroxyphenylglycol	DOPEG
3,4-dihydroxyphenylethanol	DOPET

extracellular fluid	ECF
ethylenediaminetetraacetic acid	EDTA
electroencephalogram	EEG
ethylnitrosourea	ENU
fetal calf serum	FCS
γ-aminobutyric acid	GABA
γ-aminobutyric acid transaminase	GABA-T
glutamic acid decarboxylase	GAD
gas chromatography	GC
glial fibrillary acidic protein	GFAP
hematoxylin and eosin	H + E
hydroxyeicosatetraenoic acid	HETE
5-hydroxyindole-3-acetic acid	5-HIAA
high-pressure liquid chromatography	HPLC
5-hydroxytryptamine	5-HT
homovanillic acid	HVA
intracranial pressure	ICP
intramuscular	i.m.
intraperitoneal	i.p.
intravenous	i.v.
locus coeruleus	LC
monoamine oxidase	MAO
3-methychloranthrene	3-MC
minimum essential medium	MEM
3-methoxy-4-hydroxyphenylglycol	MHPG (MOPEG)
methylnitrosourea	MNU

3-methoxy-4-hydroxyphenylethanol	MOPET
methylprednisolone	MP
magnetic resonance imaging	MRI
3-methoxytryramine	MTA
noradrenaline	NA
sodium-potassium adenosine triphosphate	Na^+-K^+-ATPase
nicotinamide adenine dinucleotide	NAD
normetanephrine	NM
β-phenylethylamine	PEA
positron emission tomography	PET
phosphotungstic acid hematoxylin	PTAH
regional cerebral blood flow	rCBF
regional cerebral glucose utilization	rCGU
regional cerebral oxygen utilization	$rCMRO_2$
regional oxygen extraction ratio	rOER
ribonucleic acid	RNA
Rous sarcoma virus	RSV
succinic semialdehyde dehydrogenase	SSADH
ventricular fluid pressure	VFP
3-methoxy-4-hydroxy-mandelic acid (vanillylmandelic acid)	VMA
volume pressure response	VPR

CHAPTER 1 REVIEW OF THE LITERATURE

1.1 Introduction

The effects of an intracerebral tumor on the function of surrounding brain, and thus on the patient's neurologic status, influence decisions concerning overall management as well as specific antitumor therapy. Most patients with brain tumors improve, albeit in a limited fashion, within 24 h after starting steroid therapy; but, the biochemical events leading to their impaired function, and the mechanisms leading to their improvement, are poorly understood.

In the following sections, the feline 9L glioma model used in this study will be compared with other experimental models of brain tumors. This review then describes the known effects of tumors on cerebral metabolism in tumor-bearing brain and the effects of dexamethasone (DEX) on these changes. The potential role of neurotransmitter alterations on functions of tumor-bearing brain and the early effects of steroid therapy are stressed.

1.2 Brain Tumor Models

Glial-origin tumors account for 45% of all intracranial tumors (Zülch & Weschler, 1968). Despite improvements in neurosurgery, radio- and chemo-therapy, 90% of patients with malignant gliomas (glioblastoma multiforme) die within 2 years (Walker et al., 1978).

Although this is primarily due to tumor regrowth, edema of the cerebral white matter contributes significantly to the mortality and morbidity in these patients. The pathophysiology of peritumor brain metabolism and associated edema is difficult to study in humans. Serial imaging, which represents the most effective method for assessing tumor growth and peritumor edema, often is difficult to achieve, and imaging to obtain control data must be performed in separate groups of patients or normal volunteers. Testing of experimental drugs can be done only under exceptional circumstances and tissue sampling to evaluate correlative biochemical changes caused by the tumor and associated edema is restricted.

Peritumor brain edema is mainly vasogenic (Klatzo, 1967). This type of edema is characterized by increased brain water and sodium content, increased permeability of capillary endothelial cells to macromolecules such as albumin, and an expanded extracellular space occupied by protein-rich edema fluid. A widely used experimental model for vasogenic edema consists of a small freezing lesion to the surface of the brain. The origin of the resultant edema, however, does not strictly correspond to peritumor edema because of cortical tissue damage and release of tissue factors, which may contribute to edema formation. Thus, definitive studies of peritumor brain metabolism and edema require the development of suitable animal brain tumor models.

The ideal experimental model of human brain tumors should have the following characteristics (modified from Bigner, 1977):

1) The tumor should be glial in composition.

2) The tumor should grow in vivo and be serially transplantable to the animal brain.

3) Tumor take should be predictable, reproducible and uniformly fatal within a reasonable period of time.

4) Survival span should be long enough to permit therapy well after the implantation procedure.

5) The tumor should behave similarly to human brain tumors in its response to chemotherapeutic drugs.

5) The animal's brain size should be adequate for diagnostic studies using imaging methods such as magnetic resonance imaging (MRI), x-ray computerized tomography (CT) and positron emission tomography (PET).

6) The animal's brain size should be adequate for biochemical studies of discrete brain areas.

There are several different ways of producing brain tumors in experimental animals; each has advantages and disadvantages depending on the nature of the study for which the model is considered.

1.2.1 Virally induced brain tumor models

Various types of viruses are able to induce transformation of brain cells when injected intracranially. The DNA viruses, adenovirus and papovavirus groups, and the RNA viruses, primarily the retrovirus group, are all able to induce brain tumors (Bullard & Bigner, 1982).

The most commonly used virus is the avian sarcoma virus (ASV). The type of tumor produced depends on which brain region has been injected with the virus suspension. In the rat and dog, 100% anaplastic astroytomas can be produced if virus injection is limited to the cortex or subependymal plate (Bigner et al., 1972, 1975; Copeland, 1975). If the dose of inoculum is standardized there is a high success rate; however, onset of clinical signs does not usually occur for 1-2 months. Tumors induced by ASV have been produced in larger laboratory animals such as dogs. Newborn beagle puppies were injected with ASV, with tumor latency ranging from 1-4 months and a success rate between 80-97% (Bigner et al., 1972, Groothuis et al, 1981). This model has been used successfully for studies involving diagnostic imaging (Groothuis et al., 1981).

Human adenovirus 12, simian adenoviruses 7 and 20 and avian adenoviruses have induced tumors in hamsters, mice, and rats with success rate between 8-93% and latency between 1-9 months. Tumor induction with papoviruses such as the JC virus or Rous sarcoma virus (RSV) has been successful in hamsters, rats, rabbits and monkeys but not dogs and cats (reviewed by Saris & Bigner, 1985).

JC virus has been inoculated into various types of monkeys with success rates ranging from 40-100% and a latency ranging from 4-40 days (London et al., 1978; Rieth et al., 1980;). Recently, Schmidt-Ruppin strain of RSV was implanted into adult Japanese monkeys (*Macaca fuscata*) (Tabuchi et al., 1985). With a 15-67 day latency, brain tumors were induced in 11 (73.3%) of 15 RSV-inoculated monkeys.

1.2.2 Chemically induced brain tumor models

A variety of neuro-oncogenic substances can produce neoplasia when either implanted into the brain or administered systemically. The main groups are polycyclic hydrocarbons and alkylating agents.

Polycyclic hydrocarbons such as 3-methylchloranthrene (3-MC), benzpyrene, dibenzpyrene, dibenzanthracene and dimethylbenzanthracene are effective when implanted intracranially in pellet form. Tumors have been successfully induced in mice, rats, and hamsters but have been unsuccessful in birds, guinea pigs, rabbits, and monkeys. Latency varied from 2 months to 2 years with a highly variable tumor incidence (Janish & Schreiber, 1977a). Gliomas, ependymoblastomas, meningiomas, sarcomas, or medulloblastomas were produced, depending on where the agent was placed in the brain (Zimmerman, 1959).

The most common alkylating agents used for the induction of tumors are ethylnitrosourea (ENU) and methylnitrosourea (MNU). Carcinogenesis has been reported in rats, rabbits, and dogs with very variable latency (Schmidek et al., 1971; Swenburg et al., 1972; Janisch & Schreiber, 1977b). Groups of animals with MNU- or ENU-induced tumors have not been used widely for treatment experiments due to variable and long latency, and poor localization. These models are, however, valuable for the study of tumor oncogenesis.

1.2.3 Transplanted brain tumor models

These models include all tumors capable of being sequentially transplanted regardless of the original induction method.

There are four major types of transplantable brain tumors:

1) The murine ependymoblastoma cell line derived from tumors induced by 3-MC (Zimmerman & Arnold, 1941).
2) The 9L, RG2, and C-6 cell lines derived from tumors induced by either MNU or ENU (Wescher et al., 1972; Chelmicka-Schorr et al., 1980; Weizaecker et al., 1981).
3) The non-central nervous system (CNS)-derived tumor cell lines such as the L1210 leukemia model, the Walker 256 carcinoma cell line, and the VX carcinoma cell line (Chirigos & Humphreys, 1966; Skipper et al., 1966; Ushio et al., 1977; Morgan et al., 1982).
4) The human glial cell lines derived from human brain tumors (Bigner et al., 1981).

The ependymoblastoma cell line grows very successfully in mice (Ausman et al., 1970); however, ependymoblastomas are very rare tumors in humans (less the 1% of the CNS tumors) and this limits the applicability of this model to brain tumor research (Zülch, 1965). Recently a rabbit model using transplanted VX-2 carcinoma has been used for research using CT scanning and radiation therapy (Cochran et al., 1985). There was a high rate of tumor induction and a short latency period (8.5 days), although there was some variability in tumor size on a given day after tumor implantation.

The nitrosourea-induced tumors 9L and RG2 have been used for many in vivo and in vitro studies, and the response to chemotherapeutic drugs is very similar to that seen in human tumors (Rosenblum et al., 1980; Weizaecker et al., 1981). In Fischer 344 rats the success rate is usually in excess of 90% with a latency of about 2 weeks (Weizaecker et al., 1981). These tumors have also been induced in non-immunosuppressed cats (Hossmann et al., 1979, Bayens-Simmonds et al., 1988). Injection of 5 x 10^5 9L glioma cells resulted in signs of increased intracranial pressure (ICP) 18-20 days after implantation (79% success rate) while injection of 1 x10^6 cells resulted in symptoms 14-16 days later (93% success rate) (Bayens-Simmonds et al., 1988). Induction of the 9L tumors into rabbits was not successful (Boisvert, personal communication).

Human glial origin cell lines have been now successfully transplanted into nude mice and rats and immunosuppressed rats and cats (Bigner et al., 1981; Maruo et al., 1982; Saris et al., 1984; Kruschelnecky, personal communication).

1.2 Summary

There are many models for experimental studies of brain tumors; however, small animal models (mouse, hamster, rat) have serious limitations for the study of peritumor brain metabolism. Poor differentiation between grey and white matter, and small brain size, make study with most imaging modalities difficult and limit both number and selectivity of biochemical analyses of regional tissue. These problems can be largely overcome by the use of larger animal hosts such as the cat, but this drastically reduces the choice of models.

Virally induced and transplantable brain tumors in cats, dogs and nonhuman primates fit most of the criteria for an ideal brain-tumor model: glial origin; growth *in vitro* with serial transplantation to the animal brain; tumor 'take' predictable, reproducible, and uniformly fatal within a reasonable period of time; survival long enough to permit therapy; response to chemotherapeutic drugs similar to that of human brain tumors; and brain size adequate for diagnostic imaging and regional biochemical studies. Additionally, virally induced tumors are authochthonous (i.e. originating in the place where found) and thus avoid the disadvantages inherent in transplanted models: the necessity of invasive implantation of tumor cells, and the possibility of tumor rejection in xenogenic hosts. Limitations of these models are: the long and variable latency of the tumors, which increases animal maintenance costs and hampers planning for experiments.

Transplantable tumors in cats appear to be the most cost-effective model for study of peritumor brain metabolism because the tumors can be implanted stereotactically with high success and short latency, allowing tissue sampling at specific times. The feline 9L glioma model may be especially suitable for neurochemical studies of peritumor brain tissue.

1.3 Effects of Brain Tumor on Brain Function

1.3.1 Definition

Brain tumors are, in essence, foreign transplants into the brain. They evoke pathological reactions in the surrounding brain by various mechanisms, the two most important being: the mass effect of the growing tumor and changes in intracranial pressure, and the cerebral edema around the tumor that aggravates the mass effect. The importance of these mechanisms will be explored and their impact on brain function will be discussed.

1.3.2 Mass effect and intracranial pressure

The connection between cerebral circulation and intracranial pressure (ICP) is based on the principles of the Monroe-Kellie doctrine (reviewed by Langfitt, 1982). The cerebrospinal fluid (CSF), blood and brain in the cranial cavity are in a reciprocal quantitative relationship. An increase in the mass of brain matter, e.g. a growing brain tumor, must lead to a decrease in volume of blood or CSF. Up to an ICP of about 40 mm Hg the brain can maintain its oxygen supply at normal levels by reactive vasodilation and increased oxygen extraction from blood (Miller et al., 1971). As ICP rises, ischemia and hypoxia develop, and once intracranial pressure approximates systemic blood pressure, cerebral circulation ceases.

Intracranial pressure is quantified by measuring CSF pressure at the levels of the cisternae magna or lumbar fossa. As the mass of the brain expands, the CSF fluid becomes more compressed. After a certain point even small changes in brain volume will cause large increases in CSF pressure. This is of clinical importance since rapid changes in pressure indicate a life-threatening situation.

Brain tumors cause a mass effect in 3 ways: growth of the tumor itself, intracranial hemorrhage in or adjacent to the tumor, and/or increases in peritumor edema.

Malignant gliomas may cause symptoms for only a few weeks before massive increases in ICP occur whereas benign tumors such as astrocytomas may cause symptoms for years (reviewed by Cobb & Youmans, 1982). The onset of a mass effect is also dependent on the location of the tumor. Medulloblastomas in the fourth ventricle grow to impede the flow of CSF, leading to hydrocephalus and increased ICP, while tumors in the cerebral cortex can grow very large before significant increases occur (Raimondi et al., 1967). Hemorrhage is common in or around gliomas and can lead to a sudden increase in ICP with resultant deterioration of the patient (Bitoh et al., 1984).

1.3.3 Brain edema

Brain edema aggravates the mass effect of the tumor, thus contributing to the development of neurological deficits and intracranial hypertension.

Peritumor brain edema is primarily vasogenic. Vasogenic brain edema was first defined by Klatzo in 1967 as edema resulting from increased permeability of the blood-brain-barrier (BBB) (Klatzo, 1967). It is characterized by:

1) An increased brain water and sodium content.
2) An extracellular space expanded by a plasma filtrate containing plasma protein.
3) An increased permeability of brain capillary endothelial cells to macromolecules.

The BBB is a structural and physiological barrier. Brain capillary endothelial cells are joined by tight junctions, and do not normally exhibit pinocytotic transport of proteins. Amino acids move through the endothelial cells by active transport, but larger peptides are not transported. Structural malformations in intratumor cerebral blood vessels are the source of BBB disruption. These malformations include fenestrations, wide intercellular junctions, an increase in pinocytotic vesicles and infolding of luminal surfaces (Long, 1970; Hirano & Matsui, 1975; Waggener & Beggs, 1976). The pericapillary glial endfoot barrier, which forms an important part of the BBB, is incomplete. In consequence, arterial hydrostatic pressure from arteries within the tumor drives protein-rich fluid into the extracellular spaces of the tumor and surrounding brain.

In peritumor edema, the increase in water content is greatest in white matter adjacent to the tumor; in white matter more remote from the tumor there is a corresponding decrease in water content (Hossmann et al., 1979; Castro, 1986; Bayens-Simmonds et al., 1988). Cortical grey matter generally has a normal water content or is only slightly edematous.

Histologically, neurons in the area of edema appear structurally normal whereas swelling is most marked in glial cells and there is also increased space between neurons and astrocytes (Szymas & Hossmann, 1984a). Astrocytes exhibit increased pinocytosis, probably due to uptake of protein. Brain tumors also induce increased levels of glial fibrillary acidic protein (GFAP) (Szymas & Hossmann, 1984b).

Peritumor brain edema may not only be associated with an absent BBB in the tumor. Phillipon et al. (1984) found that the secretory-excretory activity within meningioma tumor cells is closely associated with the production of peritumor edema. Leukotrienes, known to induce brain edema, are seen in high concentrations in brain gliomas (Black et al., 1987).

1.3.4 Clinical Neurologic Status

Neurologic signs and symptoms due to cerebral tumors may be due to:

1) Impingement of the tumor on a important structure such as a nerve tract.
2) Associated cerebral edema causing increases in intracranial pressure.
3) Compression of vital structures.

Signs and symptoms associated with cerebral edema progress as ICP rises (reviewed by Berndt, 1982; Langfitt, 1982). Headaches, often the first complaint, are perceived as intense and diffuse in character, and frequently accompanied by vomiting. Psychological changes, when they occur, are characterized by loss of interest, indifference, lack of spontaneity and affective conditions. These changes then progress to impaired memory, disorientation, and inability to concentrate; later, there may be periods of disturbed consciousness.

Papilledema is one of the most common signs of cerebral edema although its absence does not rule out increased ICP.

As brain edema and ICP increase, portions of the brain become wedged into the tentorium and foramen magnum. The third cranial nerve is stretched by transverse displacement of the brainstem or compressed by herniation of the temporal lobe. Pupillary dilatation on the side of the lesion is later followed by bilateral pupillary dilatation and loss of the pupillary reflex. Further compression of the midbrain is reflected by "extension spasms" of the extremities.

Pains in the shoulder and neck region are indicative of early cerebellar tonsillar herniation into the foramen magnum. As the medulla oblongata becomes displaced downward into the foramen magnum, vital centers become compressed. Coma, loss of tone, respiratory paralysis, circulatory disturbances, and bilateral mydriasis are indicative of the "bulbocerebral syndrome". This leads to the death of the patient through cerebral anoxia by either respiratory paralysis or circulatory disturbances (reviewed by Langfitt, 1982).

Neurologic changes seen in patients with brain tumors may not be due only to cerebral edema. In a small clinical study, neurologic function was normal in four patients although there was marked focal edema as determined by CT (Penn, 1980b). White matter edema was produced in cats using the direct serum infusion model (Nakamura et al., 1985). It was concluded that brain edema with about 80% white matter water content did not directly cause neurologic signs. These studies suggest that factors other than cerebral edema per se may contribute to the depressed neurologic state seen in patients with brain tumors.

1.3.5 Neurophysiology

In 1936, Grey Walter noted activity below 8 cpm (delta waves) in the electroencephalograms (EEG) underlying brain tumors (Walter, 1936). More recently, Hossmann et al. (1979) found that there was slowing of the EEG with appearance of high voltage 1-3/s delta waves in the affected hemisphere of 5/13 tumor-bearing cats. These EEG changes did not correlate with the duration of tumor growth, the water or electrolyte content of grey or white matter, the size of the tumor, the blood flow or intracranial pressure. Some clinical studies have shown that patients with significant peritumor edema or signs of increased ICP demonstrate hemispheric slowing of the EEG (Matsuoka et al., 1978; Kawase et al., 1985). However, Gaustaut et al. (1979) found polymorphic delta activity in brain tumors that was not specifically associated with peritumor edema observed with CT scannning.

Somatosensory evoked potentials were normal in three patient with small brain tumors and white matter edema in the somatosensory cortex (Penn, 1980a); in contrast, a patient with a mass lesion in the somatosensory cortex exhibited delayed response, suggesting that the tumor itself, not the peritumor edema, may be the cause of these changes.

Evoked potentials sometimes change in other brain insults that have associated edema. After infusion of serum into the white matter of cats (an experimental model of vasogenic brain edema) there was significant reduction of the N_1 amplitude with no delay in peak latencies of P_1 and N_1 even when grey matter regional cerebral blood flow (rCBF) and ICP were normal (Tanaka et al., 1983). These functional changes could be attributed to the mechanical distortion of the cortical layer due to expansion of the underlying edematous tissue. In experimental models with significant grey matter edema there is a correlation between a decrease in intracortical blood flow and a decrease in the direct cortical response (Nagao et al., 1985). This suggests that cortical edema or mechanical distortion are responsible for the suppression of cortical neuronal function.

Although Sutton et al. (1980) found no changes in evoked potential after production of white matter edema due to cold lesion, potentials were delayed following induction of white matter ischemia (with associated white matter edema). This suggests that ischemia, not the associated vasogenic edema, interferes with axonal functioning.

1.3.6 Cerebral blood flow and oxygen utilization

Brain tumors may also alter rCBF and oxygen utilization ($rCMRO_2$). Imaging with PET has revealed that in grey matter directly adjacent to the tumor there was an area of increased rCBF and decreased rCMRO$_2$ (Kawase et al., 1985; Tsubokawa et al., 1985), indicating an area of luxury perfusion with vasoparalysis. Slightly further from the tumor, grey matter rCBF was decreased with corresponding decreases in rCMRO$_2$ and normal regional oxygen extraction ratio (rOER) (Ito et al., 1982; Taki et al., 1985; Tsubokawa et al., 1985). This matched reduction in rCBF and rCMRO$_2$ implies that there is primary tissue metabolic failure in this area. In experimental studies, rCBF was decreased adjacent to the tumor (Hossmann et al., 1979,1986).

Regional CBF was also decreased in peritumor white matter (Hossmann et al., 1979, Ito et al., 1982., Taki et al., 1985). Hossmann et al. (1979) felt that the decrease in rCBF was due to increased water content, causing volume expansion, rather than an increased local tissue pressure, causing compression of the microcirculation, as had been suggested by Hadjidimos et al. (1971) and Reulen et al. (1972).

In the contralateral hemisphere of the tumor-bearing brain, rCBF and rCMRO$_2$ were decreased with a corresponding increase in the rOER (Ito et al., 1982; Beaney et al., 1985; Taki et al., 1985; Mineura et al., 1987). These changes were suggestive of brain ischemia. Some reasons postulated for this contralateral decrease in blood flow and oxygen consumption were:

1) Increased ICP associated with some brain tumors.
2) Transneuronal depression ("crossed cerebral diaschisis").
3) General "toxic " effect of the tumor on cerebral metabolism.

Decreases in rCMRO$_2$ have been seen in patients without significant increases in ICP (Ito et al., 1982; Beaney et al., 1985), suggesting that an increase in ICP is not the only factor. Shenkin et al., (1958) demonstrated that, in six patients with brain tumor, CBF and O_2 metabolism did not improve when ICP was lowered with direct ventricular drainage. Regional CBF and $rCMRO_2$ increased in some patients when tumor size was reduced either through surgery or radiation (Mineura et al., 1987). This suggests that either transneuronal depression or a "toxic" effect of the tumor are responsible for the remote changes in the brain.

1.3.7 Cerebral energy utilization

Cerebral energy utilization may be affected by the presence of a brain tumor. In grey matter directly bordering the tumor there were increases in glucose metabolites indicative of luxury perfusion (Tsubokawa et al., 1985). In the rest of peritumor grey matter rCGU was suppressed compared to the contralateral grey matter (DeLaPaz et al., 1983; Tsubokawa et al., 1985).

DiChiro et al. (1982) also noted that there was localized metabolic suppression in contralateral cortical areas that were neurally connected with the tumor region. This was not simply due to edema because there was a 20-60% decrease in rCGU but only a 10% increase in water content. Regional CGU was also depressed bilaterally in experimental studies (Hossmann et al., 1982, 1986; Blasberg et al., 1986). Hossmann et al. (1986) suggested that metabolic inhibition was not specific to brain tumors but was a nonspecific expression of diaschisis because it had been seen in other types of hemispheric lesions such as stroke and brain injury (Reivich *et al.*, 1977; Pappius *et al.*, 1980).

In cats with intracranial abscesses, blood flow was normal in adjacent white matter despite considerable edema (Bothe *et al.*, 1984). Energy metabolism was disturbed with increased glucose content, increased NADH/NAD$^+$ ratios and decreased adenosine triphosphate (ATP) levels. The rise in NADH/NAD$^+$ ratio suggests that the metabolic disturbance was not due to a mitochondrial deficiency, but instead to a decrease in *cellular* oxygen availability, possibly due to an increase in the diffusion distance of oxygen across the enlarged extracellular space (Bourke *et al.*, 1980). A similar mechanism could explain the metabolic changes seen in peritumor brain tissue.

There have been three *in vivo* studies on the levels of energy metabolites and lactate in human peritumor brain tissue. Two studies found decreased levels of ATP and creatinine phosphate (CrP) and increased levels of lactate (Reulen *et al.*, 1967; Tsubokawa *et al.*, 1985); in contrast, Schmiedek *et al.* (1974) found normal energy levels but increased lactate when sample values were corrected for edema. In other experimental studies, high lactate levels were recorded in the tumor and surrounding brain tissue (Paschen *et al.*, 1987). In tumor-bearing animals with neurologic signs there were decreased regional ATP levels in peritumor white matter (Mies *et al.*, 1983). Increased production of lactate is usually considered to result from the activation of glycolysis caused by inadequate oxygen supply. Paschen *et al.* (1987) suggest that this can occur not only in hypoxic regions but also in areas in which respiration and ATP hydrolysis are not tightly coupled, e.g., in brain tissue with an impaired energy state.

1.3.8 Arachidonic acid metabolism

Cell membranes are composed of a bipolar lipid layer in which protein moleoules are interspersed. In this layer lies the active transport mechanisms for the movement of proteins and ions. When the integrity of the membrane is disrupted, phospholipase A breaks up phospholipids to form free fatty acids (Fig 1). Free fatty acids, especially arachidonic acid (20:4) and docosahexaenoic acid (22:6), are rapidly released following ischemia, electroconvulsive seizures, and other brain insults causing tissue damage (Bazan & Tureo, 1980; Gardiner et al., 1981; Tang & Sun, 1982; Rodriguez de Turco et al., 1983; Wolfe & Pappius, 1984; Politi et al., 1985).

Free arachidonic acid is oxidized via two pathways: cyclooxygenase leading to the formation of prostaglandins and lipooxygenase leading to the formation of leukotrienes, hydroxyeicosatetraenoic acids (HETE's) and lipoxins (reviewed by Needleman et al., 1986). In this process free radicals are also formed.

In experimental models of vasogenic brain edema, edema can be prevented by phospholipase A_2 inhibitors such as steroids while non-steroidal anti-inflammatory drugs such as indomethacin and aspirin (cyclooxygenase inhibitors) generally have little effect (Chan & Fishman, 1978; Black & Hoff, 1985). Leukotrienes are known to cause vasoconstriction as well as being mediators of immediate

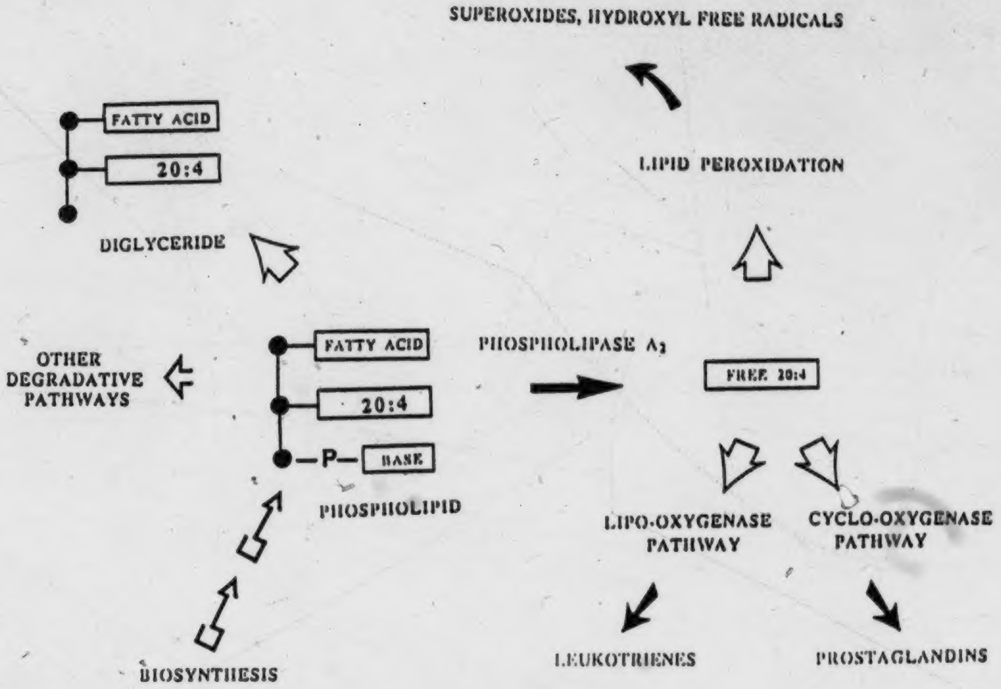

SUPEROXIDES, HYDROXYL FREE RADICALS
LIPID PEROXIDATION
FATTY ACID
20:4
DIGLYCERIDE
OTHER DEGRADATIVE PATHWAYS
FATTY ACID
20:4
P
BASE
PHOSPHOLIPID
PHOSPHOLIPASE A_2
FREE 20:4
LIPO-OXYGENASE PATHWAY
CYCLO-OXYGENASE PATHWAY
LEUKOTRIENES
PROSTAGLANDINS
BIOSYNTHESIS

Fig. 1. Arachidonic acid metabolism (modified from Pappius & Wolfe, 1984)

hypersensitivity reactions (Michelassi et al., 1982; Samelsson, 1983). Free radicals are molecules which have a lone electron in the outer orbit. This makes them highly reactive with cell membranes. They are normally inactivated rapidly by antioxidants such as vitamin E. When present in large amounts, however, free radicals may damage DNA, proteins and lipids, thus contributing to the pathogenesis of brain edema (Chan et al., 1984).

There has been very little work done on the arachidonic acid or leukotriene levels of brain tumors and surrounding brain. Black et al. (1986) found that leukotriene levels were increased in brain tumors and peritumor brain tissue compared to control tissue, with a significant correlation between brain edema and tissue leukotriene levels. One of the polyunsaturated acids in brain tumors is arachidonic acid; areas of necrosis in brain tumors may provide a focus for additional arachidonic acid production and resultant formation of leukotrienes (Wolleman, 1974). Because leukotrienes significantly increase BBB permeability when injected directly into brain parenchyma, their presence suggests their importance in peritumor brain edema (Black, 1984).

1.3.9 GABA metabolism

Various amino acids such as γ-aminobutyric acid (GABA) have been recognized as important neurotransmitters in various parts of the CNS (reviewed by Cooper et al., 1986). Imbalances in the levels or turnover of CNS GABA are implicated in the etiology of diseases such as idiopathic epilepsy (Bossi, 1986).

GABA is formed by the decarboxylation of glutamate via the enzyme glutamic acid decarboxylase (GAD). After GABA is released into the synaptic cleft by nerve impulses, it is preferentially taken up by glial cells where it is broken down into succinic acid by GABA-transaminase (GABA-T) followed by succinic semialdehyde dehydrogenase (SSADH). In addition to producing GABA, this pathway represents an alternative to oxidative decarboxylation for α-oxoglutarate metabolism--the GABA shunt.

GABA is present in inhibitory interneurons of the spinal cord, cerebellum and nigro-striatal complex and mediates both presynaptic and postsynaptic inhibition (Barber et al., 1978; Fonnum et al., 1978). There is strong evidence that GABA is concentrated within the inhibitory interneurons of the neocortex (Ribak, 1978; Hendry & Jones, 1981; Somogyi et al., 1981), although GABA-containing cells from the caudal part of the hypothalamus also project diffusely to the neocortex (Nagai et al., 1983; Vincent et al., 1983).

As would be expected by its inhibitory action, a depletion of cerebral GABA neurons or levels may lead to seizure activity (Ribak et al., 1979). Increases in cerebral GABA levels are generally seen in conditions of hypoxia (Wood et al., 1968; Norberg & Siesjö, 1975b), and also rise substantially after 30 min of global ischemia, gradually returning to normal after reperfusion (Folbergrova et al., 1974; Erecinska et al., 1984). Levels are thought to increase during ischemia and hypoxia because GAD, the enzyme responsible for GABA synthesis, can operate at maximum velocity anaerobically whereas GABA-T, the enzyme responsible for degradation, is dependent on 2-oxoglutarate and NAD+, both of which decrease in hypoxia and ischemia (Folbergrova et al., 1974; Nordstrom & Siesjö, 1978). Extracellular levels of GABA are increased in both in vivo and in vitro situations, with levels returning to normal after reoxygenation (Hauptman, 1984; Hagberg, 1985).

Only one study has measured GABA levels in and around localized cerebral infarcts (Jellinger et al., 1978). Marked elevations of GABA were seen in recent infarcts and perifocal grey and white matter, whereas intact cortex remote from the infarction and in the contralateral cortex had normal to slightly reduced GABA levels. In cold lesion brain insult, GABA levels were reduced in the injured hemisphere for one week post-insult (Ogawa, 1987). This reduction was probably due to dilution by edema fluid since brain edema in the injured hemisphere resolved during the same time period. Injection of GABA agonists into the visual or somatosensory cortex lead to defects in saccadic eye movements and in manipulative arm movements, suggesting that pathological increases in cortical GABA levels may affect cognitive function (Hikosaka et al., 1985; Newsome et al., 1985).

Levels of GABA in both experimental and human glial brain tumors are much lower then in normal brain (Mokrasch, 1971; Lefauconnier et al., 1976; Shibasaki et al., 1979). Shibasaki et al. (1979) found that gliomas had the highest levels of GABA; in gliomas, glioblastomas had the highest, astrocytoma intermediate and oligodendroglioma the lowest levels. Uptake of GABA was markedly lower in human gliomas that in normal white matter, indicating a modification in GABA metabolism in malignant tumors (Zanchin et al., 1983). Frattola et al. (1985) found a high density of [^{3}H] GABA binding sites with GABA-A receptor characteristics in human gliomas; no [^{3}H] GABA binding was detected in tumors of nonglial derivation (meningiomas, neuromas). These workers had no explanations for the possible functional role of [^{3}H] GABA binding sites in tumors of glial derivation.

In the only study describing changes in peritumor GABA, 3-MC induction of brain tumors caused an increase in GABA levels in the hypothalamus and hippocampus during the carcinogenic period (Von Metzlar & Nitsch, 1981). An inhibitor of GABA-T, aminooxyacetic acid, increased GABA content in the brain and appeared to increase the rate of tumor induction. Animals implanted with Walker carcinomas showed a trend to increased GABA levels in these regions (Von Metzlar & Nitsch, 1981).

These changes in GABA levels, as well as those induced by hypoxia and ischemia, suggest that the presence of a brain tumor and the probable ischemia associated with it may cause increased brain GABA levels.

1.3.10 Neurotransmitter amine metabolism

The roles of catecholamines such as noradrenaline (NA), dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and the indoleamine 5-hydroxytryptamine (serotonin, 5-HT) and its metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) in the pathogenesis of brain injury, ischemia, and brain edema have been explored extensively. In contrast there are only a few reports which attempt to determine the interaction between brain tumors, the surrounding brain edema and any changes in monoamine and metabolite levels.

Noradrenaline and DA are synthesized through a series of steps from the amino acid tyrosine (Fig 2). The rate-limiting step in this synthesis is the activity of the enzyme tyrosine hydroxylase. Noradrenaline-containing neurons are restricted to the pontine and medullary tegmental regions; the most important group being the locus coeruleus (LC). A group of noradrenergic fibres (medial forebrain bundle) arise from this area and continue rostrally into the frontal pole of the hemisphere where they arch caudally to supply the entire frontal, dorsal and lateral neocortex. Neurons of the LC are thought to be involved in the following functions:

1) They exert an influence on the cerebral microcirculation by increasing capillary permeability to small molecules and by regulating blood flow and water homeostasis (Raichle et al., 1975; Bates et al., 1977), although this has been disputed by Dahlgren et al. (1981).
2) They elicit a rise in heart rate and systemic blood pressure, possibly by regulating vasopressin release (Lightman et al., 1984).
3) They inhibit activity in most target neurons (reviewed by Amaral & Sinnamon, 1977).
4) They are activated by stressful, threatening stimuli, and that stimulation may produce behavior characterized by fear. Stimulation may also selectively enhance or diminish the effect of neurotransmitters released from other cells; in general the function of the LC may be to continously monitor the environment

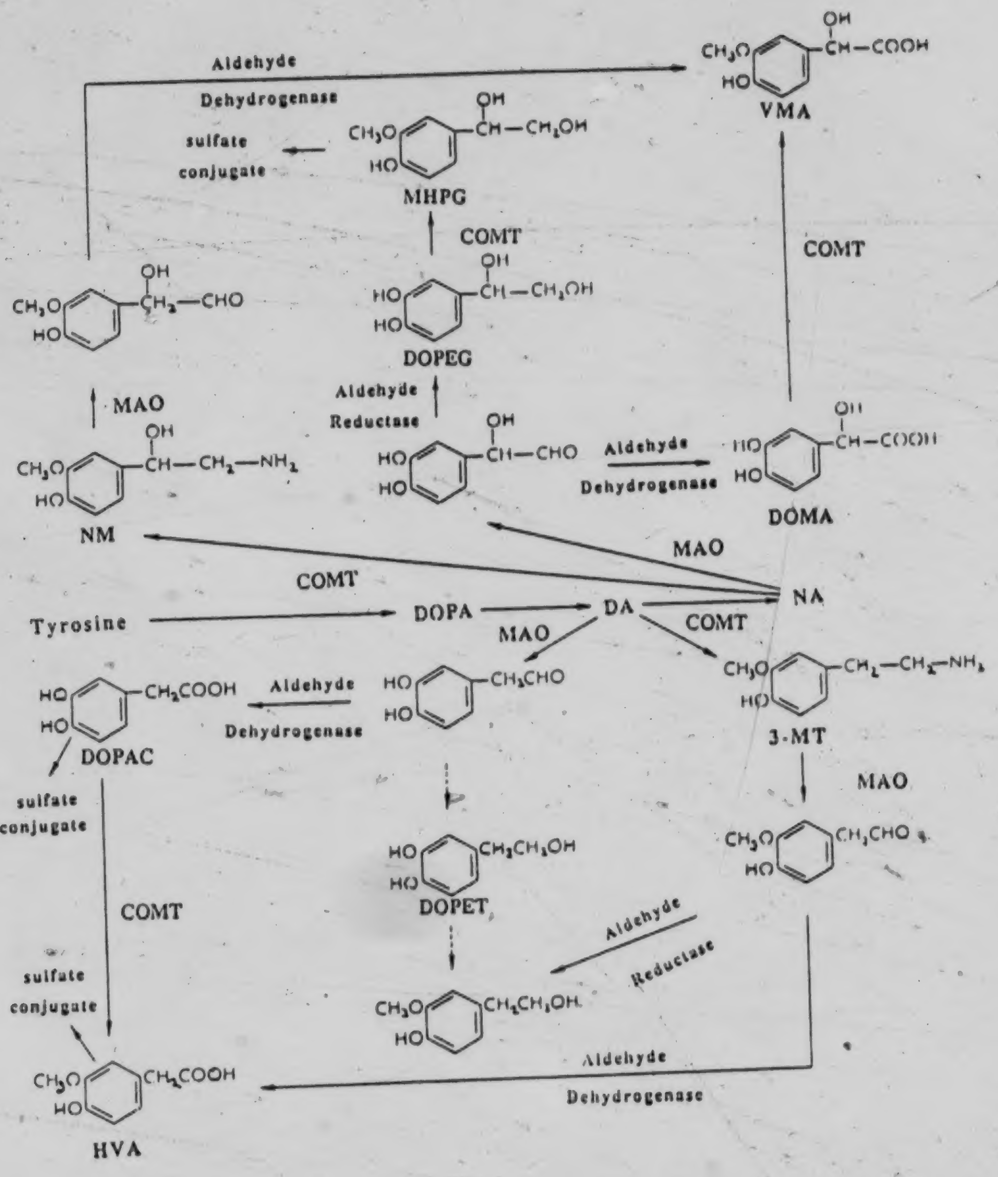

Aldehyde Dehydrogenase
VMA
sulfate conjugate
MHPG
COMT
COMT
DOPEG
MAO
Aldehyde Reductase
Aldehyde Dehydrogenase
NM
DOMA
MAO
COMT
NA
Tyrosine
DOPA
DA
MAO
COMT
Aldehyde Dehydrogenase
DOPAC
3-MT
sulfate conjugate
MAO
COMT
DOPET
Aldehyde Reductase
sulfate conjugate
Aldehyde Dehydrogenase
HVA

Fig. 2. Flow chart illustrating the synthesis and metabolism of NA and DA (modified from Cooper *et al.*, 1986).

and prepare the organism to cope with emergencies (reviewed by Neiwenhuys, 1985).

The monoamine DA is not only a precursor of NA but also a neurotransmitter in its own right. Dopaminergic neurons originate from the cell groups A9-10 (ventral tegmental area) in the midbrain and project to the medial frontal lobe, entorhinal cortex and the anterior cingulate cortex in the rat (Lindvall et al., 1974). Other areas such as the temporal and parietal cortex appear to be innervated in the cat (reviewed by Oades & Halliday, 1987).

Dopamine may have several roles in cortical function. Iontophoresis of DA onto cortical neurons leads to slow and relatively long-lasting reductions in spontaneous discharge (reviewed by Moore & Bloom, 1978). The mesocortical neurons are also implicated in the control of cognitive processes, with lesions causing learning and attention impairment in delayed alternation tasks (Simon et al., 1979). This system is also activated (increased turnover) during stressful situations (Blanc et al., 1980; Herman et al., 1982). Dopamine may also act indirectly on cortical neurons; given i.v., DA increases cerebral blood flow, coupled to focal increases in glucose utilization (Herstad, 1981).

The indoleamine 5-HT is synthesized from the precursor amino acid tryptophan with the rate-limiting enzyme being tryptophan hydroxylase (Fig 3). Serotonergic neurons going to the neocortex originate from cell groups B6-B8 in the cranial brainstem. They ascend through the medial forebrain bundle to distribute evenly thoughout the neocortex. In the monkey, various cortical areas may

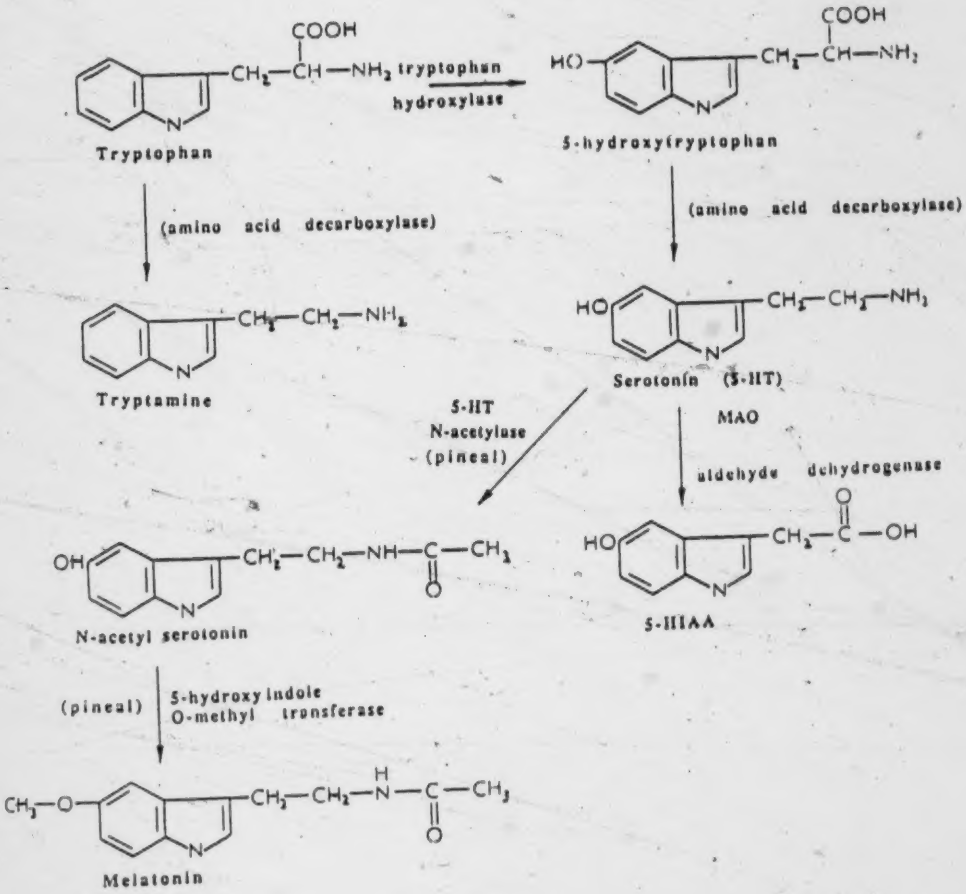

COOH
CH₂—CH—NH₂
tryptophan hydroxylase
Tryptophan
HO
COOH
CH₂—CH—NH₂
5-hydroxytryptophan
(amino acid decarboxylase)
(amino acid decarboxylase)
CH₂—CH₂—NH₂
Tryptamine
HO
CH₂—CH₂—NH₂
Serotonin (5-HT)
5-HT N-acetylase (pineal)
MAO
aldehyde dehydrogenase
OH
CH₂—CH₂—NH—C—CH₃
O
N-acetyl serotonin
HO
CH₂—C—OH
O
5-HIAA
(pineal)
5-hydroxy indole O-methyl transferase
CH₃—O
CH₂—CH₂—N—C—CH₃
H
O
Melatonin
N

Fig. 3. Flow chart illustrating the synthesis and metabolism of 5-HT (modified from Cooper et al., 1986).

show considerable differences in density and layering of serotonergic fibres (Takeuchi et al., 1983).

Projections of 5-HT fibres to small intraparenchymal blood vessels in the brain may be involved in the regulation of blood flow. Many neurons terminate in the ventral tegmental area and LC, and it has been suggested that these 5-HT neurons may have an inhibitory influence on NA and DA neurons from these areas. In addition, serotonergic neurons may act to regulate the flow of CSF by modulating the motility of ependymal cell cilia (Aghajanian et al., 1975).

The presence of the tumor or associated peritumor edema may change levels of neurotransmitters in the brain. Alternatively, altered levels of extracellular neurotransmitters may directly cause brain edema. If brain monoamines were altered due to the tumor there could be direct effects on neurological function.

In brain insults with cytotoxic edema (i.e., increased uptake of water into cells with little or no increase in extracellular water content) there were profound changes in monoamine and metabolite levels. After triethyl-tin poisoning, decreases in NA, DA, and 5-HT levels occur in various brain regions, with concomitant increases in HVA and 5-HIAA (Bentue-Ferrer et al., 1985). These changes point to an increased release of monoamines, probably due to decreased membrane integrity. Increased levels of metabolites may be due to increased turnover of monoamines or a result of decreased blood flow which prevents washout of the metabolites.

In cytotoxic brain edema associated with Reye's syndrome, decreased levels of NA and DA were found in various parts of the brain (Lloyd et al., 1977). This could be representative of increased release of monoamines as increased levels of ventricular and plasma monoamines have been seen in children affected by this syndrome (Faraj et al., 1984; Arcinue et al., 1986). Both ischemia and epilepsy have important components of cytotoxic edema and show similar depletions in monoamines and increases in metabolites (Mrsulja et al., 1976; Jellinger et al., 1978; Matsumoto et al., 1984; Baran et al., 1987).

High extracellular levels of NA and 5-HT may in themselves cause cell damage and further edema formation. Depletion of NA levels or inhibition of 5-HT synthesis have led to decreased edema and improvement of cerebral metabolism in experimental models of ischemia (Welch et al., 1976; Maruki et al., 1982; Busto et al., 1985). Early studies seemed to point to a role of NA and/or 5-HT in the formation of brain edema. An application of 5-HT to the surface of the brain resulted in increased BBB permeability and formation of brain edema (Osterholm et al., 1967; Mohanty et al., 1979). But, a later study by Doczi et al. (1984), in which 5-HT was injected intraventriculary, failed to show any effect of 5-HT on brain water content. In vitro studies failed to show any effect of 5-HT or DA on cell swelling; NA induced astrocytic swelling (Bourke et al., 1983).

A cold lesion causes vasogenic brain edema which is most similar to that seen in peritumor brain. Pappius & Dadoun (1987) found that there was a decrease in hemispheric 5-HT 24 h after lesioning, with a subsequent return to normal levels. Increases in 5-HIAA lasted longer, being significantly different from controls 4 h as well as 1, 3, and 6 days after the lesion. Abnormalities in 5-HT turnover were more pronounced in portions of the cortex close to the lesion. Alterations in 5-HT metabolism may contribute to functional depression of brain after a cold lesion; inhibition of 5-HT synthesis by ρ-chlorophenylalanine ameliorated the decrease of cortical rCGU seen in traumatized brain (Pappius & Wolfe, 1983a). Noradrenaline levels were also decreased bilaterally after a cold lesion although levels of DA and its metabolites were unchanged (Pappius & Dadoun, 1986, 1987). In contradiction, an earlier study by Dave & Dastur (1985) showed increased levels of NA and no changes in 5-HT in edematous gyri 3 h after a cold lesion. In other forms of CNS trauma (whiplash, spinal cord injury), increased, decreased, and unchanged levels of monoamines have been reported (Osterholm et al., 1971; Nafchi et al., 1974; Boismare et al., 1985).

Very little experimental work has been done on the relationship between brain tumors and changes in peritumor monoamine metabolism. Levels of monoamines and their metabolites have been reported to be low both in human and experimental tumors (Ikeda & Natazawa, 1984; Ikeda et al.,1984); in other clinical studies, alterations in CSF 5-HIAA and HVA levels did not correlate with the type of brain tumor (Ikeda & Nakasawa, 1981).

Two small, fairly limited, studies have been done to study the effect of a brain tumor on peritumor monoamine metabolism. Chang et al. (1988) found decreases in cortical NA and striatal DA in rats implanted with 9L glioma tumors, although changes in cortical NA may have been related to the cortical edema observed. Von Metzlar & Nitsch (1985) found that transplanted (Walker carcinoma) and chemically induced brain tumors (3-MC) caused decreases in NA, DOPAC, and 5-HIAA in various brain regions; DA, 5-HT, and per cent water content were not measured. These observations suggest that brain tumors do alter peritumor brain monoamine metabolism although the mechanism remains undetermined.

1.3.11 Summary

Brain tumors exert profound effects on peritumor metabolism. These may be due to the tumor itself, the mass effect and changes in ICP, the associated brain edema, or poorly understood effects such as altered arachidonic acid, GABA, or neurotransmitter amine metabolism. Clinical and experimental studies show depressed grey-matter metabolism both adjacent to, and far from, the tumor. Although edema plays a significant role in these changes, studies show that rCBF, O_2 metabolism, rCGU, and neurotransmitter levels are altered even in areas where edema is not significant.

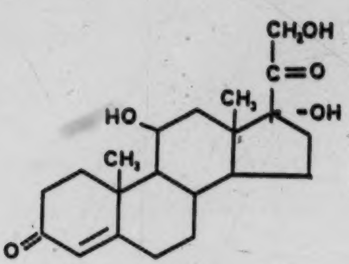
The monoamines NA, DA, and 5-HT play important roles in brain function both by selectively inhibiting or exciting cortical neurons and by regulating CBF, and water and CSF homeostasis. In addition, neurons containing these neurotransmitters interact with neurons projecting to other brain regions (i.e., glutamatergic neurons going to the striatum) and GABAergic cortical interneurons. Changed monoamine and GABA levels, a reflection of altered function, are present in neurologic diseases such as ischemia and epilepsy. Measurement of neurotransmitter amines and GABA in peritumor brain is a first step in determining whether altered neurotransmitter function is a major factor in the etiology of depressed peritumor brain metabolism.

1.4 Dexamethasone: Structure and Properties

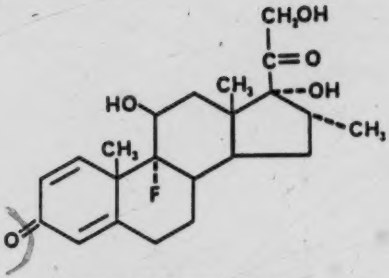
Dexamethasone (DEX) is a synthetic analog of the naturally occurring glucocorticoid cortisone. Cortisone is secreted by the adrenal cortex in a circadian fashion. In humans, morning values are the highest, with late evening secretion the lowest (Carpenter et al., 1971); this rhythm is reversed in nocturnal animals such as the cat (Krieger et al., 1968).

Cortisone and its analogs are composed of three fused 6-carbon rings and one 5-carbon ring. Dexamethasone and methylprednisone (MP)possess a C1-C2 double bond which is absent in cortisone. Dexamethasone also has a fluorine at position 9 and a methyl group at position 16, enhancing the anti-inflammatory action while decreasing the mineralocorticoid effect (Fig 4). The biological half-life is about 12 h for hydrocortisone and cortisone, 12-36 h for prednisone and MP, and 36-54 h for DEX (Swartz & Dluhy, 1978).

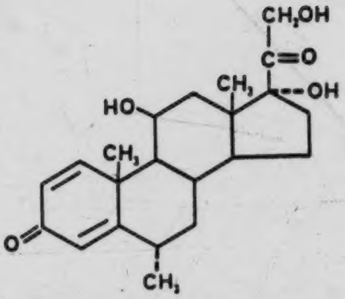
Naturally occurring glucocorticoids stimulate gluconeogenesis in the liver and reduce extrahepatic protein and fat stores. Synthetic glucocorticoids are usually employed for their anti-inflammatory, not gluconeogenic, properties. In most organs steroids decrease capillary permeabilty, thus decreasing edema formation. The passage of leukocytes and macrophages into traumatized areas is impeded by a steroid-induced decrease in local endothelial sticking (Allison et al.,


CH₂OH
C=O
CH₃
HO
-OH
CH₃
O

CORTISOL


CH₂OH
C=O
CH₃
HO
--OH
CH₃
CH₃
F
O

DEXAMETHASONE


CH₂OH
C=O
CH₃
HO
--OH
CH₃
O
CH₃

METHYLPREDNISOLONE

Fig. 4. Structures of cortisol, dexamethasone, and methylprednisolone

1965). Glucocorticoids also seem to inhibit cell membrane injury, probably by preventing lipid peroxidation caused by arachidonic acid metabolites (Braughler, 1985). Demopoulos et al. (1972) proposed that glucocorticoids may do this by intercalating between unsaturated fatty acids in the cell membrane, thus shielding the susceptible carbon double bond in the unsaturated fatty acid from free radical attack.

Steroids are bound in varying degrees to plasma protein; but only free steroid crosses the BBB. Interactions with individual cells involves two mechanisms: uptake of steroid into the cell and its interaction with cytosolic receptors, and interaction of the steroid with receptors on the cell membrane.

When steroid enters a cell it binds to a free cytosolic receptor, leading to transformation of the receptor-steroid complex. This modulates gene expression which then leads to changes in amino acid incorporation, specific protein formation, and induction of characteristic enzymes, thus bringing about a target cell response to hormone action. This mechanism of action is slow in onset and lasts long after the drug has disappeared from the body.

Steroids may also interact with the cell at the level of the cell membrane prior to, or independent of, hormone-receptor effects mediated via gene expression (Baulieu, 1978; Sadler & Maller, 1982). These actions are rapid in onset, short in duration and occur in parallel with the rise and decline of hormone levels in the tissue (McEwen et al., 1978).

Although DEX is somewhat similar in action to cortisone, the pattern of uptake in the brain is different. In normal rat brain 50.2 ±

3.7% of an administered dose of [^{3}H]-DEX was found equally divided in the cerebral hemispheres, with the cerebellum and brain stem showing about the same amount of labelled DEX (24.2 ± 5.1%, 24.8 ± 3.0%) (Meaney & Aitken, 1985). Seventy-five percent of DEX was distributed to astrocytes, 25% to neurons. Cortisone receptors are concentrated primarily in the limbic system (primarily hippocampus) and in motor neurons of the cranial nerves as well as dispersed in the reticular formation. In contrast, DEX receptors have an even distribution over the brain, including the cortex (McEwen et al., 1976).

Data showing the effects of cortisone or hydrocortisone on brain function should be interpreted with caution since cortisone and DEX may work through different receptor sites and DEX may even block cortisol receptors, at least in the hippocampus (reviewed by McEwen et al., 1986).

1.5 Effects of Dexamethasone on Function of Normal Brain

1.5.1 Intracranial pressure

A number of investigators have reported significant reductions in CSF production ($\leq$ 50%) when glucocorticoids were administered to normal experimental animals (Garcia-Bengochea, 1965; Sato, 1967; Johnson et al., 1975; Weiss & Nulson, 1976).

1.5.2 Clinical neurologic status

Administration of exogenous glucocorticoids, including DEX, may produce psychiatric disturbances such as depression, psychosis, or mania in patients (Ling, 1981; Carpenter & Gruen, 1982; Sovner, 1983). Patients with Cushing's disease often exhibit psychiatric symptoms (Carpenter & Gruen, 1982). Psychotic depression is a subgroup of depressive diseases in which patients may exhibit extremely high post-DEX cortisol levels (Schatzberg et al., 1983). Schatzberg et al. (1985) hypothesized that prolonged endogenous hypercortisolemia could be associated with the development of psychosis in depressed patients.

The effect of glucocorticoids on animal behavior is complex. Adrenalectomy facilitated extinction of a food-reinforced straight runway response (Micco & Ewan, 1979), impaired the forced extinction of a learned passive avoidance response (Bohus & DeKloet, 1977, 1981) and reduced exploratory behavior (Veldhuis et al., 1982). Replacement with physiological doses of corticosterone reversed these effects but DEX administration was ineffective. Most dosages of DEX given to patients are, instead, in the pharmacological range; behaviour changes seen in patients may reflect DEX effects on membrane receptors or speed of synaptic transmission, rather then receptor-mediated action on limbic system function.

1.5.3 Brain water homeostasis

Studies of the effects of steroids are generally done by investigating adrenalectomized animals. Adrenalectomy in rats caused significant increases in cerebral water and Na^+ content which were prevented by aldosterone or glucocorticoids, including DEX (Baethmann et al., 1972). Later, studies using adrenalectomized dogs showed that increases in water and Na^+ content were restricted to cerebral grey matter (Baethmann et al., 1982). Administration of DEX did not change water content in normal brain; however withdrawal of DEX led to increased water content (Hedley-White & Hsu, 1980). This may have been due to DEX-induced changes in BBB permeability.

Little work has been done on the effect of steroids on normal brain water content and Na^+/K^+ levels. In rats, chronic administration of ACTH, cortisone, corticosterone, dehydrocorticosterone, or 11-desoxy-17-hydroxycorticosterone had no effect on brain electrolytes (Woodbury et al., 1954).

Sodium-potassium adenosine triphosphatase (Na^+-K^+-ATPase) is important in the maintainence of cell water homeostasis. Cortisone and MP induced Na^+/K^+-ATPase activity in the brain (Stastny, 1971; Braughler et al., 1981). Induction occurred within minutes of intravenous administration, and the time course followed closely the accumulation and later disappearance of MP in the tissue (Braughler et al., 1981).

1.5.4 Neurophysiology

Acute administration of glucocorticoids may affect brain excitability and neuronal transmission. Intrathecal administration of prednisone or hydrocortisone produced seizures in dogs after a 30 min latency (Oppelt & Rall, 1961). The steroid effect may also be biphasic; low-dose prednisolone increased susceptibility to strychnine-induced seizures while very high doses (100 mg/kg) had the opposite effect (Mansor et al., 1956). In clinical studies, Glaser (1953) reported seizures in patients with Cushing's disease, and Glaser & Merrit (1952) showed an increased seizure rate in epileptic patients treated with steroids. Suprisingly, adrenalectomy has also been associated with enhanced seizure susceptibility (Torbati et al., 1975).

Steroids may cause single cell excitation or inhibition, depending on the type of cell and the brain region studied (Ruf & Steiner, 1967, Phillips & Dafny, 1971; Dafny et al., 1973). Intravenous cortisol administration increased the amplitude of evoked potentials in the medial lemniscus, midbrain reticular formation, and thalamic nuclei (Feldman et al., 1970) whereas adrenalectomy caused a decreased amplitude and prolonged conduction of multisynaptic evoked potentials (Feldman et al., 1961).

Steroid-induced changes in neuronal excitability have been closely studied in the lumbar spinal reflex pathways and in alpha motor neurons. There were rapid dose-related increases in spinal monosynaptic transmission after i.v. injection of high doses

(≥ 15 mg/kg) of MP (Hall & Baker, 1979). Acute administration of 30 mg/kg resulted in a resting hyperpolarization and slowed initial segment conduction; conduction velocity was increased in the rest of the axon, indicative of facilitated myelinated axonal conduction. The excitability (mean synaptic response) of the motor neuron was also increased (Hall, 1982). These effects were seen when high pharmacological doses of steroids were given and probably reflected membrane interactions rather then cytosolic receptor interaction. This could explain the contradictory effects on brain excitability seen in steroid-treated and adrenalectomized (steroid-deficient) animals.

1.5.5 Cerebral blood flow and oxygen utilization

A study by Baethmann et al. (1979) found that, in dogs, acute adrenalectomy without steroid substitution significantly decreased CBF and oxygen uptake. Cerebral blood flow was normalized by aldosterone replacement while oxygen consumption was normalized by addition of DEX.

1.5.6 Cerebral energy utilization

Dexamethasone did not change rCGU in normal rats (Pappius et al., 1982). For ethical reasons clinical PET studies have lacked control groups treated with DEX.

1.5.7 Arachidonic acid metabolism

Large amounts of free arachidonic acid are only released after various brain insults (see chapter 1.3.7); therefore, the action of DEX on arachidonic acid metabolism will be discussed in the section dealing with the effect of DEX on arachidonic acid in tumor-bearing brain (see chapter 1.6.7).

1.5.8 GABA metabolism

Evidence suggests that DEX administration changes GABA content or turnover in the CNS. Incubation of brain slices with hydrocortisone increased tissue levels of GABA, possibly through inhibition of GABA-T (Banay-Schwartz et al., 1979). In another study, however, levels of GAD and GABA-T in rat brain were unchanged by adrenalectomy (Edwards et al., 1980); administration of DEX to intact rats also had no effect on these enzymes.

Glucocorticoids may also have effects on GABA receptors. The steroids cortisone and MP enhanced $GABA_A$ receptor affinities in the cerebral cortex, cerebellum, thalamus and hippocampus (Majewska et al., 1985); suprisingly, DEX, which is very similar to MP, had no effect. The authors hypothesized that elevated levels of glucocorticoids, recorded in stressful situations, may act as natural anticonvulsants, thus protecting neurons from over-stimulation. This may be true in selected brain regions because DEX and other glucocorticoids either cause neuronal excitation or inhibition, depending on the area studied (see chapter 1.5.3).

Inhibition of GAD prevented DEX-induced decreases in blood cortisone levels (Acs et al., 1978), suggesting that GABA may be involved in the mechanism of DEX inhibition of resting- or stress-induced release of adrenocorticotrophic hormone (ACTH) from the pituitary. Administration of DEX may prevent stress-induced decreases in monoamines and increases in GABA recorded in various brain regions (Blanc et al., 1980; Reinhard et al., 1982; Yoneda et al., 1983).

1.5.9 Neurotransmitter amine metabolism

Reports vary on the effect of DEX and other steroids on nervous tissue levels of NA and DA. Generally, most groups found increases in DA in various CNS regions (Hellstrom et al., 1979; Hall & McGinley, 1982; Yanase et al., 1984; Rothschild et al.,1985) although some groups reported either no effect or a decrease in NA levels (Hall & McGinley, 1982; Rothschild et al., 1985; Bayens-Simmonds et al., 1986). Effects of steroids may be biphasic; Hall & McGinley (1982) found that spinal cord levels of DA and 5-HT were increased one h after MP administration; but, 24 h later, NA, DA, and 5-HT levels were all significantly decreased.

Dexamethasone is thought to increase the synthesis of NA and DA. It is known to enhance trans-synaptic induction of tyrosine hydroxylase by cold stress, reserpine treatment, cyclic AMP (cAMP), nerve growth factor or acetylcholine (Hanbauer et al., 1975, Otten &

Thoenen, 1977; Thoenen & Otten, 1978; Tank & Sun, 1982). Glucocorticoids may also directly induce tyrosine hydroxylase activity (Williams et al., 1981; Tank & Ham, 1984), perhaps through a second messenger system (Brostrom et al., 1974; Haycock et al., 1982; Forray et al., 1985).

Levels of DA and possibly NA may be increased in the synaptic cleft by inhibition of presynaptic uptake or decreased catabolism by monoamine oxidase (MAO). Gilad et al. (1987) found that MP reduced DA uptake into synaptosomes. Although adrenalectomy increased MAO activity (Clarke & Sampath, 1975), administration of DEX to intact animals did not decrease MAO activity in different brain regions. However, indirect evidence (changes in DOPAC/DA and 5-HIAA/5-HT ratios) suggests that DEX may inhibit MAO activity (Hellstrom et al., 1979; Rothschild et al., 1985).

Post-synaptic receptor numbers and/or response may also be increased by steroids. DEX increased the number of post-synaptic β-adrenergic receptors in astrocytoma cell cultures (Foster & Harden, 1980), although Roberts & Bloom (1981) found that adrenalectomy also increased the Bmax of β-adrenergic receptors in the hippocampus.

It is difficult to evaluate the effect of DEX on regional 5-HT and 5-HIAA levels since most of the literature focuses on the effect of adrenalectomy and replacement therapy with corticosterone. Adrenalectomy decreased tryptophan hydroxylase activity and 5-HT turnover in various brain regions, especially the hippocampus (Sze & Towle, 1976; Van Loon et al., 1981a, 1981b; DeKloet et al., 1982). Replacement therapy with corticosterone reversed these effects;

pretreatment with DEX negated this normalizing effect by blocking corticosterone receptors in the hippocampus (DeKloet et al., 1982). In intact animals, i.p. DEX appeared to decrease 5-HT turnover in various brain regions, including the cortex, perhaps by a blocking effect on corticosterone receptors (Kawamura et al.,1984; Rothschild et al., 1985; Bayens-Simmonds et al., 1986).

In conclusion, there are no definitive effects of DEX on brain levels of NA, DA, and 5-HT although there is evidence that treatment leads to increased synthesis and/or decreased turnover of DA, 5-HT, and possibly NA.

1.5.10 Summary

In addition to decreasing peritumor brain edema, treatment with DEX may improve depressed peritumor brain metabolism through ill-defined processes. The effect of DEX on function of normal brain was reviewed in order to elucidate its mechanism of action.

Administration of DEX is associated with psychiatric disturbances such as depression, psychosis, and mania; experimental studies indicate that DEX may increase brain excitability through various mechanisms such as increased speed of synaptic transmission. On the other hand, there is only sketchy evidence that DEX increases CBF, O_2 metabolism or rCBF in normal brain.

Dexamethasone's positive effect on neurologic function might be a result of stimulated neurotransmitter metabolism. Although GABA levels are little affected, studies indicate that neurotransmitter amine synthesis is stimulated and degradation may be inhibited by glucocorticoids, perhaps leading to increased tissue levels. Early neurologic improvements in patients with brain tumors may be due directly to this stimulatory effect and/or to DEX-induced improvements in peritumor cerebral metabolism.

1.6 Effects of Dexamethasone on Function of Tumor-Bearing Brain

1.6.1 Mass effect and intracranial pressure

Patients with brain tumors often have slightly elevated ICP, with circadian changes in plateau waves of type A (wave of increased ICP of more then 5 min duration with rapid rise and fall) and type B (rhythmic waves of increased ICP of 1/2- 1 min duration) (Brock *et al.*, 1976; Miller & Leech, 1975; Yamaura *et al.*, 1984). Increases in ICP caused by plateau waves can lead to rapid deterioration in patients due to decreased cerebral blood flow. Treatment with DEX decreases the frequency and lowers the amplitude of plateau waves ("barostabilization") within 24-48 h. Fewer and smaller fluctuations of ICP correspond to smaller fluctuations of cerebral perfusion pressure and improved cerebral blood flow.

This decrease in ICP fluctuations is partially due to a reduction in the volume-pressure response (VPR). The VPR is estimated by measuring the increase in ventricular fluid pressure (VFP) produced by a small (1 ml) increase in CSF volume in the lateral ventricle. This is a measurement of brain elastance ($\Delta P/\Delta V$) and is equivalent to inverse compliance. Seven patients with brain tumors were studied prior to and 24 h after the administration of bethamethasone (10 mg initially then 4 mg/6 h). The VFP was not significantly reduced, but the VPR, and the number of plateau waves was decreased (Miller *et al.*, 1977) and the patients improved clinically. The decrease in VPR renders the brain more tolerant to increases in brain edema, delaying large increases in ICP. The ability of DEX to inhibit CSF production or increase CSF absorption may also contribute to the early decrease in plateau waves (Garcia-Bengochea, 1966; Weiss & Nulsen, 1970).

The mass effect of the tumor itself can be decreased by glucocorticoid therapy. When given for a number of days, DEX and MP reduced the size of experimental tumors, with a concomitant decrease in peritumor edema (Gurcay *et al.*, 1971; Shapiro & Posner, 1974). *In vitro*, both DEX and MP produced inhibition of cell growth and caused cytotoxic damage in cell cultures of human malignant gliomas (Mealey *et al*, 1971). A very high dose of MP for one day (400 μg/ml) caused more damage than 3-9 days of treatment with 100 μg/ml. Clinically, high doses of MP produced decreases in apparent tumor size (measured by CT) 7 days after MP administration (Leiguarda *et al.*, 1982); neurologic improvement ranged from moderate to marked 24-48 h after initiation of therapy. During long-term steroid therapy, decreases in tumor size may contribute to neurological improvement; however, it is unlikely that this mechanism is responsible for the improvements seen in the first 24 h of therapy.

1.6.2 Clinical neurologic status

It is well known that steroid treatment improves neurological status rapidly (within hours) in patients with peritumor edema. The level of consciousness improves rapidly, although, both clinically and experimentally, brain edema and raised ICP do not resolve for at least 24 h (Miller & Leech., 1977; Miller et al., 1977; Luthert et al., 1986; Bell et al., 1987). Various hypotheses for this rapid improvement will be discussed in the following sections.

1.6.3 Brain edema

The best known effect of DEX is its ability to decrease peritumor brain edema. This has been shown in both clinical (Galicich & French, 1961; Reulen et al., 1972; Meinig et al., 1977) and experimental studies (Gurcay et al., 1971; Shapiro & Posner, 1974; Matsuoka & Hossmann, 1981; Yamada et al., 1983).

Steroids may decrease peritumor brain edema by:

1) Reducing capillary permeability in tumors.
2) Reducing capillary permeability in peritumor tissue.
3) Reducing edema spread and increasing resolution.
4) Increasing transport of Na^+/ K^+ and water across the capillary/tissue interface either in the tumor or peritumor area.
5) Inhibiting tumor growth (see chapter 1.6.1).

Glucocorticoids have been shown to reduce capillary permeability in experimental tumors (Yamada et al., 1983, 1987; Reichman et al., 1986; Nakagawa et al., 1987), human tumors (Jarden et al., 1985;

Bodsch et al., 1987), and peritumor brain tissue (Yamada et al., 1983,1987); some authors did not find an increased capillary permeability in peritumor brain (Hossmann et al., 1983). It is generally thought that capillary permeability is reduced by decreasing vesicular transport or pore size in tumor capillaries. An alternative reason was demonstrated by Bruce et al. (1987), who found that a protein product of malignant brain tumor, when injected in test animals, rapidly increased capillary vascular permeability; this effect was inhibited by pretreatment of the animals with DEX. This suggests that factors other then structural anomalies in the tumor may be responsible for edema production. Other authors have found no effect of DEX on tumor vascular permeability (Hossmann et al., 1983; Luthert et al., 1986). A similar effect has been reported in cold-lesion injury studies (Yen et al., 1985).

DEX may also reduce edema by preventing its spread through white matter or increasing its resolution. Hossmann et al. (1983) suggested that DEX prevented spread of edema by reducing the water-binding capacity of extravasated proteins because, in a feline brain-tumor model, treatment for one week with DEX led to a decrease in peritumor white matter water content without a decrease in extravasated proteins. Nakagawa et al. (1987) found that DEX treatment appeared to decrease the extracellular space both in and adjacent to the tumor; this would cause a marked decrease in bulk flow of edema of edema fluid.

A major mechanism suggested for resolution of edema is protein uptake by astrocytes (Klatzo et al., 1980). This decreases the oncotic pressure in the ECF, allowing water to move into the vascular system.

DEX did not promote uptake of protein so it must restore water and ion homeostasis despite increased extracellular protein levels. It has been suggested that DEX may act directly to increase transport of Na^+/K^+ and water across the capillary/tissue interface, although this has not been clearly demonstrated in peritumor tissue (Long et al., 1966).

Treatment with DEX decreases brain edema in peritumor brain and in other experimental models, but it is generally agreed that the edema is not decreased for at least 24 h after DEX administration (Pappius et al., 1969; Maxwell et al., 1971; Luthert et al., 1986; Bell et al., 1987). Thus, early improvement in neurological status may not be due to a reduction in brain edema.

1.6.4 **Neurophysiology**

Slow waves (delta waves) in EEG patterns are associated with brain tumors and may be related either to the tumor or associated peritumor edema (see chapter 1.3.4). In a clinical study of patients with brain tumors, Matsuoka et al. (1978) found that focal delta waves changed as early as 20 min after DEX administration in patients with clinical signs of increased ICP. They attributed this to a reduction in peritumor edema. Because studies show edema does not decrease for 24 hr after DEX, these changes may reflect an improvement in brain excitability due to a decrease in the frequency and amplitude of plateau waves and/or an early improvement in neurotransmitter/metabolic function. An early study by Pappius & McCann (1969) showed that DEX diminished EEG abnormalites measured 48 h after cold lesion injury; these changes in EEG abnormalities did not correlate with changes in brain edema. In a later study it was determined that the EEG changes were not due to edema but, instead, due to epileptic changes resulting from the cold lesion (Schaul et al., 1976). The improvement in EEG may be due to the ability of DEX to improve depressed rCGU in this model (Pappius et al., 1982).

Changes in evoked potentials after DEX administration have not been measured in patients with brain tumors; however, MP prevented decreases in cortical evoked potentials, as well as normalizing blood flow and preventing edema, in an experimental model causing cortical edema (exposure of cortex to air) (Nagao et al., 1985).

1.6.5 Cerebral blood flow and oxygen utilization

In a feline brain-tumor model, DEX treatment (10 mg i.m. total dose, given 1 week before sacrifice) prevented the decrease in peritumor white matter blood flow seen in untreated animals (Matsuoka et al., 1981) The decrease in CBF in untreated animals was proportional to the increase in water content, but treatment resulted in normal to slightly increased blood flow even though some edema was still present. Intracranial pressure was not elevated in either treated or untreated animals.

Tumor-bearing rats showing neurologic signs of increased ICP were given or not given a single injection of DEX (20 mg/kg) (Mies et al., 1983). Untreated animals showed decreased rCBF in peritumor grey and white matter; treatment led to decreased white matter edema and increased cortical blood flow. In a similar study, tumor-implanted rats were either treated or not treated with MP (15 mg/kg/day) from 5 to 10 days after tumor inoculation; on day 10 untreated rats had a 50% reduction in rCBF in the cortex of the tumor-bearing hemisphere. Methylprednisolone treated rats had significantly higher rCBF (Yamada et al., 1983).

Reulen et al. (1972) found a mean ipsilateral increase in CBF in six patients after 5-7 days of treatment with DEX. Buttinger et al. (1982) found that 50% of patients studied (n=10) had decreased rCBF before treatment. In these patients, rCBF did not increase after one day of treatment but increased in 9/20 hemispheres after 7 days of treatment. Regional CBF also increased in brain regions that had normal blood flow before treatment.

Recently, patients with brain tumors were studied with PET just prior to, and 1-5 days after institution of DEX therapy (Leenders et al., 1985). After DEX treatment, rCBF decreased in all brain regions except in edematous areas. Regional oxygen utilization remained unchanged and increases in rOER compensated for the effects of decreased blood flow. Regional CBF prior to therapy was decreased compared to control values.

All the studies showing a positive effect of DEX on CBF reported an associated decrease in brain edema; however, with treatment, rCBF increased more than brain edema decreased.

1.6.6 Cerebral energy utilization

It has been proposed that the positive early effect of DEX and other glucocorticoids on neurologic status is through improvement in cerebral energy metabolism (Pappius et al., 1984). The work done to date partially supports this. Yamada et al. (1983) showed that 5 days of treatment with MP significantly reversed the 25-40% decrease in rCGU seen in tumor-bearing cortex of untreated animals. In a shorter time study (24 h), tumor-bearing rats with neurologic signs (n=4) given a single injection of DEX (20 mg/kg) had improved white matter ATP levels, but no significant improvement in decreased rCGU (Mies et al., 1983). Improvement in rCGU due to DEX have been seen in other forms of brain injury. Dexamethasone ameliorated the bilateral depression of rCGU seen 1 day and 3 days after cold-lesicn injury (Pappius et al., 1982). Changes in rCGU in treated and untreated animals did not correlate with changes in rCBF, which was generally elevated. In addition, changes in rCGU occurred in the contralateral hemisphere, where there was no significant edema. The authors concluded that the benefical effects of DEX were due primarily to improvements in cerebral metabolism. A preliminary study by Kuchiwaki et al. (1985) tends to support this observation. A single injection of MP (30 mg/kg) was given to dogs at the time of focal infarct induction; 4 h later, the energy charge/creatinine ratio was maintained at slightly higher levels in the non-infarcted hemispheres of treated animals compared with untreated animals.

In the only clinical study to date, Schmiedek et al. (1976) found that 3 days of DEX treatment (8 mg/kg b.i.d.) did not significantly alter levels of CrP, ATP, ADP, AMP, lactate, or pyruvate in biopsies of peritumor grey matter.

These studies show mixed evidence that DEX and other steroids improve depressed cerebral energy metabolism. In the study by Yamada et al. (1983) the length of treatment (5 days) would be sufficient to decrease the size and mass effect of the tumor; this may explain the positive results of MP.

1.6.7 **Arachidonic acid metabolism**

The effect of DEX on arachidonic acid release has not been studied in brain tumors or peritumor brain tissue. Corticosteroids have an inhibitory effect on the enzyme phospolipase A_2 and subsequent release of arachidonic acid (Flower & Blackwell, 1978; Hong & Levin, 1976), perhaps by stimulating the synthesis of an inhibitory protein. Dexamethasone inhibited brain edema induced by arachidonic acid and also prevented lipid peroxidation of cell membranes (Chan, 1983; Braughler et al., 1985). The early onset of protection, and the high dose of glucocorticoid required, suggests that glucocorticoid receptors were not involved; instead, DEX probably stabilized cell membranes, thus preventing breakdown of membrane phospholipids (Demopolous et al., 1972; Braughler et al., 1985). Treatment with DEX could block the formation of high leukotriene levels in brain tumors and peritumor tissue, thus decreasing brain edema and tissue damage caused by these compounds.

1.6.8 GABA metabolism

No experimental studies have been done to evaluate the effect of DEX on GABA metabolism in peritumor brain. This would be an important area of study since DEX-induced decreases in cortical GABA levels could partially explain the early neurological improvement in patients with brain tumors.

1.6.9 Neurotransmitter amine metabolism

Only one study has attemped to determine the effect of DEX on peritumor monoamine and metabolite levels. Chang et al. (1988) found that decreased cortical NA and striatal DA levels induced by a brain tumor were prevented by 2 days of therapy with DEX (20 mg/kg/day). Improved levels of cortical NA could be a direct result of DEX on monoamine metabolism, or could be secondary to decreased brain edema in that area. This preliminary report, as well as studies in normal animals, suggest that early changes in neurological function after DEX treatment may be partially due to changes in peritumor monoamine metabolism.

1.6.10 Summary

It is clear that steroids such as DEX improve neurologic function in patients with brain tumors even in the first day of therapy. The best known property of DEX is its ability to decrease peritumor brain edema by various mechanisms: by decreasing capillary permeability within the tumor, reducing the volume of edema, or increasing the resolution of edema. Regional CBF is decreased in edematous tissue; however, DEX has been shown to increase rCBF by a greater degree then the concurrent decrease in edema. Decreased cerebral edema results in improved levels of consciousness, especially if ICP is reduced; but, because edema is not reduced in the first 24 h, this mechanism is probably not responsible for early neurologic improvements.

Early changes induced by DEX include decreased VPR, making the brain less susceptible to volume increases, and decreased number and amplitude of plateau waves, lessening the number of substantial rises in ICP.

It has been suggested that a principal early effect of DEX is stimulation/normalization of cerebral energy utilization; this hypothesis has been substantiated in several experimental studies. In addition to its effect on cellular utilization of glucose, DEX treatment may affect the metabolism of arachidonic acid, GABA and neurotransmitter amines, which may be abnormal in tumor-bearing brain. Because there is evidence of appreciable levels of arachidonic acid and leukotrienes in tumor and surrounding tissue, and because DEX is known to inhibit arachidonic acid metabolism, one action of DEX may be to decrease tissue damage caused by these factors.

Levels of GABA may be raised in peritumor brain, perhaps leading to some inhibition of cognitive function. Dexamethasone may normalize GABA levels directly, or indirectly by increasing oxygen levels to neurons. Lastly, DEX is known to have direct effects on neurotransmitter amine synthesis and/or degradation. If neurotransmitter amine levels are depressed in peritumor brain, as has been suggested, DEX may cause early clinical neurologic improvement by improving monoamine metabolism.

1.7 General Summary

As this review of the literature has demonstrated, there is considerable evidence that brain tumors affect brain metabolism. In addition to its well known ability to increase ICP, displace adjacent tissue, and produce brain edema, the tumor may also depress brain metabolism in both adjacent and remote areas. Changes in brain neurotransmitter levels have been demonstrated in many other neurologic conditions. A more complete understanding of neurotransmitter alterations in peritumor brain would aid in the design of treatment to reverse these abnormalities.

Dexamethasone has been used for more then 20 years in the treatment of patients with brain edema, yet, investigators do not agree on its mechanisms of action. It causes mood elevations and increased seizure susceptibility in healthy people, possibly through increased neuronal excitability and/or stimulation of neurotransmitter amine synthesis; also, neurologic improvements in patients with brain tumors exceed its ability to decrease brain edema. The cause of this generalized and rapid improvement has been the subject of much speculation and study. Besides decreasing brain edema, DEX has been shown to decrease VPR and plateau waves and to improve rCBF and rCGU. It also inhibits arachidonic acid synthesis, preventing the formation of leukotrienes, known to cause edema. Another postulated action, little studied, may be the ability of DEX to normalize GABA and neurotransmitter amine levels in peritumor brain, possibly leading to improvements in neurologic status.

CHAPTER TWO MATERIALS AND METHODS

2.1 Experimental Animals

Male and female mature random-source cats (2.2-6.0 kg) used in Studies A, B, C, and D were obtained from Health Sciences Laboratory Animal Services, University of Alberta, Edmonton, Alberta. They were housed singly in laboratory cat cages. The cats were subjected to controlled conditions of 12-h on/off lighting schedule and constant temperature control (21°C). Food and water were provided ad libitum. The cats were fed Feline Maintenance Cat Food (Hill's Pet Products, Inc., P.O. Box 148, Topeka, Kansas 6601, U.S.A.) composed of 30% crude protein (min), 20% crude fat (min), 2% crude fibre (max), 10% moisture (max), 5% ash (max), 0.6% calcium (min), 0.5% phosphorus (min), and 0.1% magnesium.

2.2 Brain Tumor Model

2.2.1 Culture of 9L-glioma cells

9L-glioma cells were obtained from the Brain Tumour Research Centre, University of California, San Francisco, CA, U.S.A. They were stored frozen at -70°C in a minimum essential medium (MEM) containing 5% fetal calf serum (FCS), 500 i.u. penicillin/ml, 500 μg streptomycin/ml, 20 mmol L-glutamine/ml, with 10% dimethyl sulfoxide (DMSO). Two days before injection the suspension was thawed at room temperature and the cells were placed in MEM + 5% FCS without DMSO until they were in a rapid growth phase. The monolayer was then incubated in 2.5% trypsin containing 20 mg ethylenediaminetetraacetic acid (EDTA) at 37°C for 10 min; this was followed by a second incubation in fresh solution. The cells were then removed and placed in 15 ml of MEM with 5% FCS, counted using a hemacytometer, and resuspended in media to the desired concentration. This cell suspension was stored at 4°C and used within 6 h.

2.2.2 Preparation

All cats had their food removed the afternoon before anesthesia (approximatly 18 h). They had free access to water.

2.2.3 Anesthesia

Cats were anesthetized with atropine (0.3 mg/kg), xylazine (2 mg/kg), and ketamine (20 mg/kg).

2.2.4 Surgery

The head was placed in a head holder and the dorsal surface was shaved and aseptically prepared for surgery. A midline skin incision was made, the right temporalis muscle was partly reflected laterally and the sagittal and coronal sutures were identified. A 3-4 mm diameter craniectomy was made in the cranium 5.5 mm lateral to the sagittal suture and 5.5 mm caudal to the coronal suture. Using a 50 µl Hamilton microsyringe placed in a Brinkmann compact micro-manipulator, 50 µl of cell suspension or medium alone was injected 5.5 mm below the surface into the central white matter (Fig. 5). The cells were injected over 2 min and the needle was left in the brain for 2 min following injection so that the cells would disperse and not flow up the needle tract. The needle was slowly withdrawn and the bone defect filled with bone wax before closing the skin incision. Recovery in all animals was uneventful.

2.2.5 Post-operative care

Cats were maintained on a heating pad until they were in sternal recumbency; usually one h after initiation of anesthesia. Animals were observed once/h for 6 h after anesthesia and allowed free access to food one day later. Animals were observed at least once daily for signs of infection and neurologic symptoms of increased ICP; if there was anorexia or abnormal behaviour, rectal body temperature was monitored and neurologic assessment done. Procaine penicillin was administered once daily and the surgical site cleaned if there was evidence of localized infection at the skin incision. Animals with evidence of neurologic deficit were sacrificed on the day that they reached a neurologic score of 2. If animals became

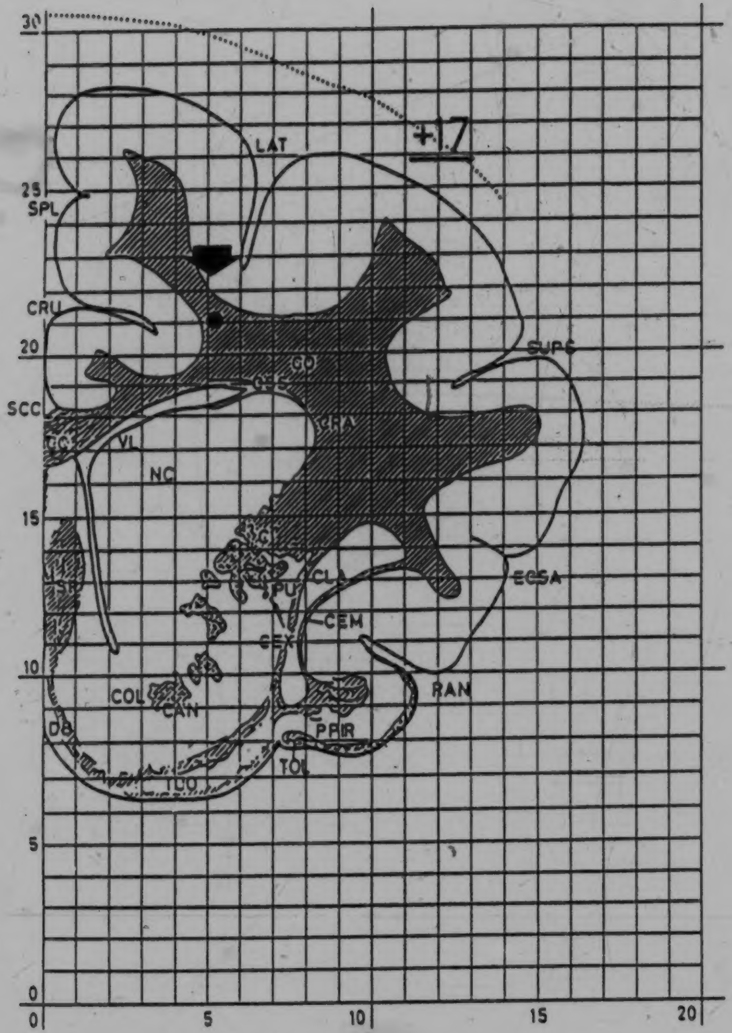

30
25
SPL
CRU
20
SCC
15
10
5
0
LAT
+17
SUPS
GO
CRA
VL
NC
PU
CLA
ECSA
CEM
CEX
RAN
COL
CAN
DB
PPIR
TOL
TUO
0
5
10
15
20

Fig. 5. Site of tumor cell implantation. Coronal section of cat brain at coordinates +17, Horsley-Clarke method. (modified from Reinoso-Suaréz, 1961).

dehydrated, they received subcutaneous lactated Ringer's solution; however, no animals received DEX unless indicated in the protocol. All animals were cared for in accordance with the guidelines of the Canadian Council of Animal Care.

2.3 Administration of Dexamethasone

2.3.1 Schedule 1 (DEX 1)

Tumor-bearing and control cats (n=6/group) were given DEX (0.25 mg/kg i.v. and 0.25 mg/kg i.m.) with 0.25 mg/kg i.m. repeated in 6 h. Other groups of control cats (n=6/group) were not treated or treated with vehicle alone (0.1% sodium metabisulphite, 0.15% methylparaben and 0.02% propylparaben in distilled water) at the same dosage schedule as DEX 1. Animals were sacrificed 2 h after the second dose of DEX or vehicle.

2.3.2 Schedule 2 (DEX 2)

Tumor-bearing and control cats (n=6/group) were given DEX (0.25 mg/kg i.v., 0.25 mg/kg i.m.) with 0.25 mg/kg i.m. given 3 additional times at 6 h intervals. Another group of control cats (n=6) were treated with vehicle alone at the same dosage schedule as DEX 2. Animals were sacrificed 2 h after the second dose of DEX or vehicle.

2,4 Clinical Examination

2.4.1 Neurologic Assessment

Immediately before sacrifice or first drug administration, cats were assessed for neurologic evidence of increased ICP according to a standardized scoring system (Table 1). Depression is defined as lassitude and decreased interest in surroundings with ability to respond normally to stimuli. Somnolence is defined as lassitude and decreased interest in surroundings with decreased response to stimuli. Stupor refers to an animal which is responsive only to deep pain stimuli. Animals were considered to have neurologic signs of increased ICP if they had somnolence and anisocoria (grade 2 on the scoring system). These animals were used on the day that they exhibited these signs. They were assessed immediately before the first administration of DEX 1 dosage schedule and every 2 h after until they were anesthetized for *in vivo* freezing.

2.4. Measurement of intracranial pressure

Intracranial pressure was measured immediately prior to *in vivo* freezing in anesthetized animals. It was measured by inserting a saline-filled 20 g needle into the cisternae magna. The needle was then connected via saline-filled tubing to a Tektronix Blood Pressure monitor. Confirmation of the presence of the needle in the subarachnoid space was done using the

NEUROLOGIC SCORING SYSTEM

0	No Neurologic Deficits
1	Anorexia and Depression
2	Somnolence and Anisocoria
3	Somnolence, Anisocoria, and Hemiparesis
4	Stupor, Bilateral Abnormal Pupillary Reflexes, and Hemiparesis/Hemiplegia
5	Coma

Table 1. Neurologic scoring system to determine neurologic signs of increased intracranial pressure.

Valsalva maneuver which increases ICP. The pressure increased by at least 5 mm Hg during this procedure.

2.5 Brain Tissue

2.5.1 Freezing _in situ_

Fifteen days after xenotransplantation, tumor-bearing and sham-operated animals were examined for neurologic deficits. Clinically normal animals were tranquilized with xylazine (2 mg/kg) then anesthetized with halothane, N_2O and O_2. Animals were paralyzed with gallamine triethiodide (2 mg/kg) and ventilated using a Harvard ventilator or Bourke infant ventilator. Body temperature was maintained at 37°C with a heating pad. The femoral artery was catheterized and arterial blood pressure determined using a transducer attached to a Tecktronix moniter. Blood-gas levels were measured using a Micro 13 pH/blood gas analyzer (Instrumentation Laboratory Inc). Immediately before _in vivo_ freezing, the blood gases and pH were normal: pH 7.25-7.42, pCO_2 28-36 mm Hg, pO_2 greater than 120 mm Hg. The mean arterial blood pressure was between 70-110 mm Hg. The head was frozen _in vivo_ with liquid N_2 using a modification of the method of Welsh & Rieder (1978) then removed, and stored at -30°C until dissection. Control animals were frozen using the same methods. Untreated and DEX 1-treated cats with neurologic signs were sacrificed on the day they had a neurological score of 2.

2.5.2 Regional dissection of brain tissue

The heads were thawed to 0°C and the brain removed in a cold room at 0-4°C. The brain was removed and sliced coronally at the site of tumor injection, and the presence of a tumor verified. Hemorrhage was noted, if present, and the diameter of the tumor was measured along the dorsal-ventral and medial-lateral axes. The larger measurement was considered to be the "diameter" of the tumor. Any other pathological findings, such as midline shift, were also noted.

Representative samples of tumor, peritumor (parietal) grey and white matter, frontal grey and white matter and temporal grey matter from both hemispheres (Fig. 6, 7, 8) were dissected out and either used or refrozen on dry ice. Samples were stored at -70°C until analysis.

2.6 Equipment

2.6.1 Gas chromatograph

A Hewlett-Packard (HP) 5880A gas chromatograph equipped with a 15 m narrow-bore SE-54 (1% phenylmethyl polysiloxane) capillary column (HP), a ^{63}Ni electron-capture detector, a HP 7672A automatic sampler and an HP 5880A GC terminal was employed. Helium was used as carrier gas with a flow-rate of 2 ml/min. A mixture of argon-methane (95:5) at a flow-rate of 36 ml/min was used as make-up gas. The injection port

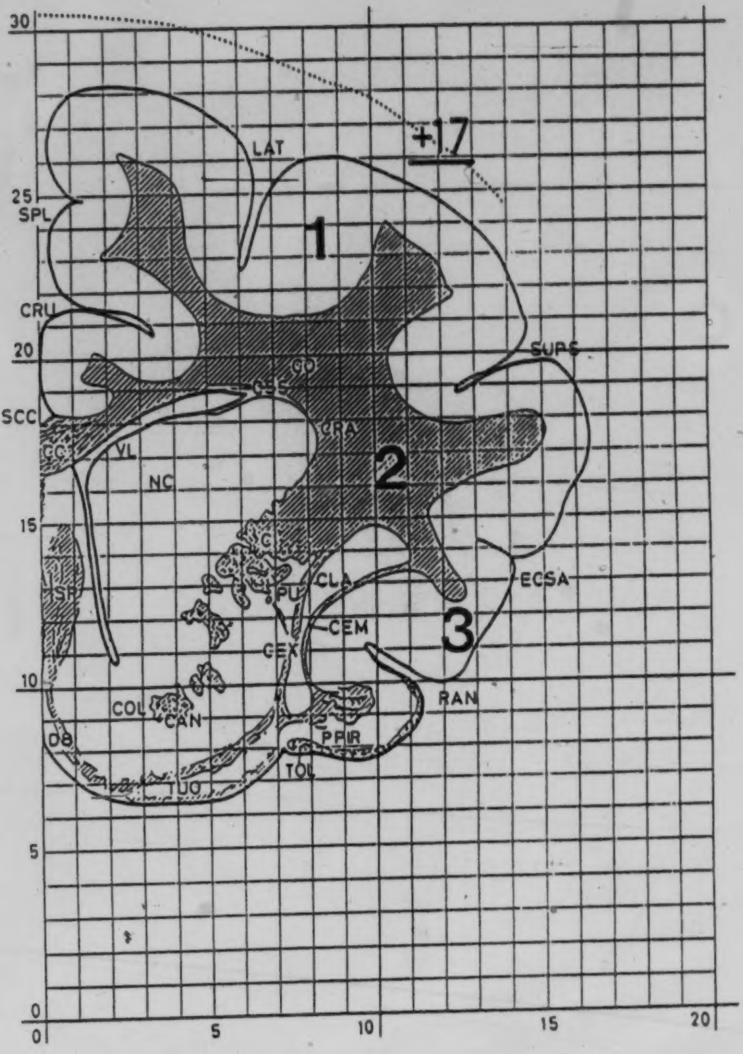

30
+17
LAT
25
SPL
1
CRU
20
SUPS
CO
CSS
SCC
CRA
CC
VL
NC
2
15
C
CLA
PU
ECSA
SP
CEM
3
CEX
10
RAN
COL
CAN
DB
PPIR
TOL
TUO
5
0
0
5
10
15
20

Fig. 6. Regional brain sampling areas at the site of tumor implantation. Coronal section of cat brain at coordinates +17, Horsley-Clarke method.
(modified from Reinoso-Suaréz, 1961).

1. parietal grey matter
2. parietal white matter
3. temporal grey matter

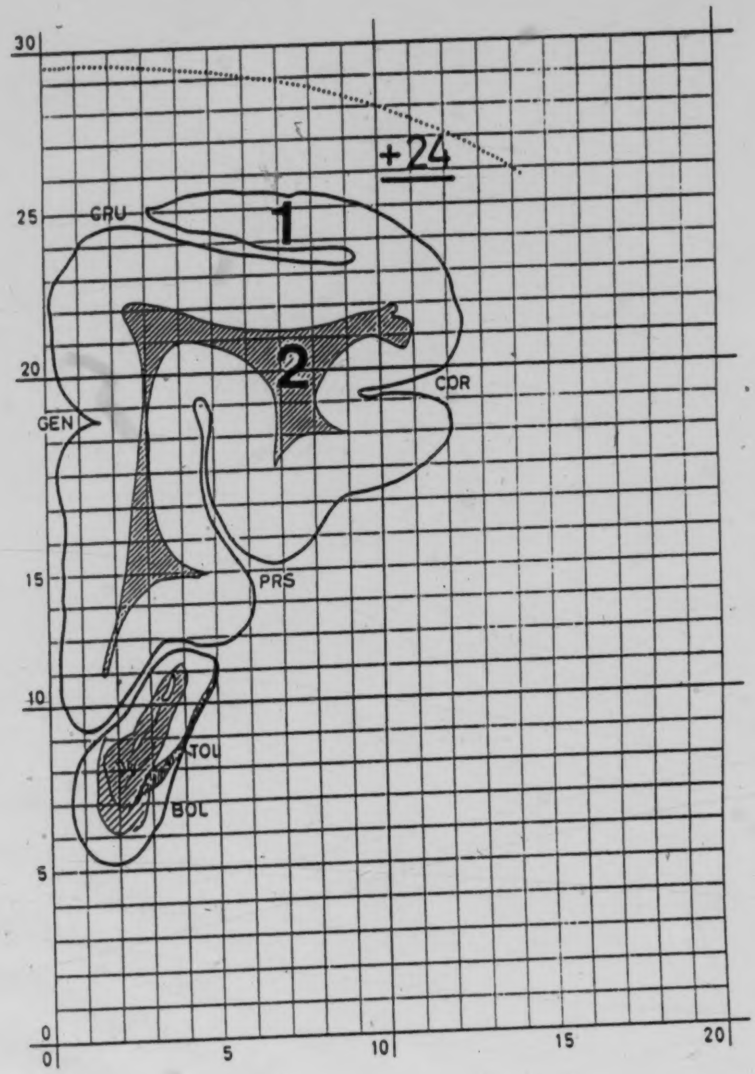

+24
1
2
GRU
GEN
COR
PRS
TOU
BOL
30
25
20
15
10
5
0
0
5
10
15
20

Fig. 7. Regional brain sampling areas in the frontal cortex. Coronal section of cat brain at coordinates +24, Horsley-Clarke Method. (modified from Reinoso-Suaréz, 1961).

1. frontal grey matter
2. frontal white matter

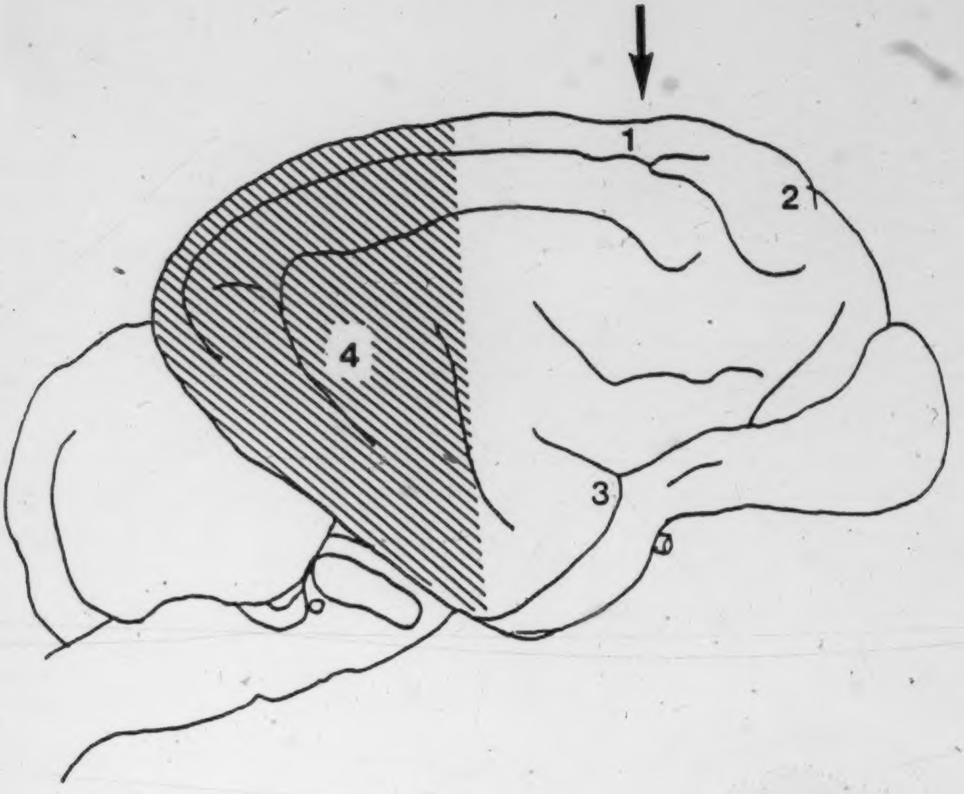


Fig. 8. Regional brain sampling areas. Lateral view of brain. (modified from Reinoso-Suaréz, 1961).
1. parietal grey matter
2. frontal grey matter
3. temporal grey matter
4. caudal cerebrum (used for MAO experiments)
→ site of tumor cell implantation

temperature was maintained at 200°C. The oven program was as follows; initial temperature of 140°C for 0.5 min, then increasing at a rate of 5°C/min to 180°C. This temperature was maintained for 0.1 min, then the temperature was increased at a rate of 8°C /min to 310°C where it was maintained for 3 min.

2.6.2 High-pressure liquid chromatograph

The apparatus consisted of a solvent delivery system (model M45, Waters Associates, Milford, MA, U.S.A.) equipped with a WISP automatic injector (Model 710B, Waters Associates, Milford, MA, U.S.A.) set to inject 20 µl. Separation was achieved on a C_{18} reversed-phase column (3.9 mm x 15 cm) (µBondapac, Waters Associates, Milford, MA, U.S.A.) in association with a C_{18} precolumn (µBondapac (Corasil), Waters Associates, Milford, MA, U.S.A). The compounds were detected on a glassy carbon electrode (Model LC17 Bioanalytical Systems (BAS), West Lafayette, IN, U.S.A.) set at 0.75 V versus a Ag/AgCl reference electrode and regulated with an amperometric detector (LC-4B Bioanalytical Systems (BAS), West Lafayette, IN, U.S.A.) with the range set at 2 nA with offset 0-10 nA.

2.6.3 Liquid-scintillation spectrometer

Ex vivo monoamine oxidase (MAO) inhibition studies were performed using a radiochemical procedure (Wurtman and Axelrod, 1963), and the radioactivity was counted using a

Beckman model LS 7500 (microprocessor-controlled) liquid scintillation spectrometer coupled to a Datamex Model 43 printer. The LS 7500 is a soft-beta counting spectrometer with 300-sample capacity and is equipped with Automatic Quench Compensation (AQC), H-Number quench monitor, keyboard operation, program editing feature, and 10-library counting programs in an unalterable memory.

2.6.4 Glassware

Glass tubes were cleaned by washing with tapwater and a solution of biodegradable Sparkleen (Fisher Scientific Co.) They were then placed in an ultrasonic cleaner (Mettler Electronics) containing a solution of Decon 75 concentrate (BDH Chemicals), 20 ml to 1 litre, and sonicated 3 separate times for 20 min intervals. The tubes were washed in an electronic dishwasher (Miele Electronic G715) in wash-rinse mode with distilled water then air-dried in a mechanical convection oven (Model 28, Precision Scientific Group).

The grinding tubes and pestles were cleaned by washing with tapwater and a solution of biodegradable Sparkleen (Fisher Scientific Co.). They were then sonicated in the same manner as the glass tubes, rinsed with tapwater followed by distilled water, and air-dried in a mechanical convection oven.

2.6.5 Block heaters

A Reacti-Therm Heating Module (Pierce Chemical Co.) or Multi-Blok Heater No. 2090 (Labline Instruments), both with adjustable temperature control, were employed in the GABA analysis procedure.

2.6.6 Balances

A Mettler AE 160 or Mettler AE 1603 was used for weighing of all biological samples or chemicals used in the various analyses. These balances were also used to weigh tissue samples in order to ascertain their percent water content.

2.6.7 Tissue homogenizers

Homogenization of brain samples weighing 150 mg or more was done at a a speed of 7,000 rpm using a TRI-R Model S63c variable speed laboratory motor with a Teflon glass pestle and a glass grinding tube (clearance: 0.1-0.15 mm).

Brain samples weighing less than 150 mg were homogenized manually using a 1.5 ml glass tissue grinder and pestle (Radnoti Glass).

2.6.8 Centrifuges

2.6.8.A Bench Centrifuge

A Sorvall GLC-2B or Sorvall GLC-1 General Laboratory Centrifuge (Dupont Instruments) was used for low-speed centrifugation.

2.6.8.B High-speed centrifuge

Centrifugation of the homogenate of brain samples ≥150 mg was performed in a Damon-IEC Model B-20 (heavy duty) refrigerated high-speed centrifuge. Samples were centrifuged at 10,000 rpm for 15 min at a temperature of 0-4°C.

2.6.8.C Microcentrifuge

The centrifuging of brain homogenate of small samples (brain samples less than 150 mg) was done in a MSE Micro-Centaur microcentrifuge. The homogenate was centrifuged at 15,000 rpm for 1.5 min at room temperature.

2.6.9 Shaker/Mixer

A IKA-VIBRAX-VXR® (Janke & Kunkel GmbH, Sweden) tube shaker was used to shake the tubes in the extraction phase of the GABA assay. A benchtop Thermolyne Maxi Mix 1® (Sybron Instruments, Dubuque, IA, U.S.A.) vortexer was used for vortexing of individual tubes.

2.7 Chemicals

All chemicals, drugs and bioactive amines used are shown in Table 2. A Corning AG-3 or Corning Mega-Pure (3 Litre Automatic) still was used to produce double-distilled water for use in analysis. The derivitizing agent, pentafluorobenzoyl chloride, neurotransmitter amines, metabolites and amino acids were stored at 0°C. Stock solutions (1 mg/ml) of neurotransmitter amines, metabolites and amino acids were stored at -20°C and replaced with fresh solutions every month.

2.8 Tissue Measurements

2.8.1 Measurement of water content

Microfuge tubes (1.5 ml, Bio plas) were pretared, then weighed with the tissue sample. The tubes were dried at 30°C for 72 h, cooled then reweighed. The water content value was expressed as:

$$\%\ \text{water content} = \left(\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \right) \times 100$$

Table 2. Chemicals

The following is a list of the names of the chemicals employed in this study and their respective suppliers or manufacturers

Chemicals	Suppliers or Manufacturers
(-)-noradrenaline (NA) HCl	Sigma Chemical Co. (St. Louis, Mo., U.S.A.)
dopamine (DA) HCl	
homovanillic acid (HVA)	
5-hydroxytryptamine (5-HT) creatinine sulfate	
3,4-dihydroxyphenylacetic acid (DOPAC)	
5-hydroxyindole-3-acetic acid (5-HIAA)	
3,4-dihydroxybenzylamine (DHBA) HBr	
epinine (EPI) HCl	
α-methyl-ρ-tyrosine	
γ-aminobutyric acid (GABA)	
methylparaben	
propylparaben	
pentafluorobenzoyl chloride (PFBC)	Aldrich Chemical Co. (Milwaukee, WI., U.S.A.)
Triton X-100	Terochem Laboratories (Edmonton, Alta., Canada)
chloroform (ACS)	BDH Chemicals Toronto, Ont, Canada
methanol (HPLC grade)	
isobutanol	
toluene (ACS)	

Table 2 (continued).

hydrochloric acid	Fischer Scientific (Fairlawn, NJ., U.S.A.)
sodium bicarbonate	
potassium chloride	
perchloric acid (70%)	
ethylenediaminetetracetic acid (EDTA)	
ascorbic acid	
decane	
isobutanol	
methanol (HPLC grade)	
sodium phosphate dibasic anhydrous	
sodium acid phosphate	
dimethylsulfoxide (DMSO)	
sodium metabisulfite	
hydroxytryptamine binoxalate, 5-[2^{14}C] (specific activity 55 mCi/mmol)	New England Nucleur (NEN) (Lachine, P.Q., Canada)
phenylethylamine HCl, 2-[ethyl-1-^{14}C] (specific activity 50.2 mCi/mmol)	
isopentane (2-methylbutane)	Eastman Kodak (Rochester, NY. U.S.A.)
octyl sodium sulfate	
dexamethasone, 4 mg/ml (Decadron Phosphate® Injection)	Merck, Sharp and Dohme Canada (Kirkland, P.Q., Canada)
gallamine triethiodide, 20 mg/ml (Flaxedil 100®)	Rhône-Poulenc Pharma Inc. (Montréal, P.Q., Canada)
xylazine hydrochloride, 20 mg/ml (Rompurn®)	Haver-Lockhart Inc. (Calgary, Alta., Canada)
ketamine hydrochloride, 100 mg/ml (Ketalean®)	M. T. C. Pharmaceuticals (Cambridge, Ont., Canada)
atropine sulfate BP, 0.6 mg/ml	Glaxo Laboratories (Toronto, Ont., Canada)

8.2 Analysis of regional brain levels of catecholamines, 5-hydroxytryptamine and metabolites

Brain regional concentrations of NA, DA, 5-HT, DOPAC, HVA and 5-HIAA were determined by high-pressure liquid chromatography (HPLC) with electrochemical detection (Kim et al., 1983). The mobile phase was composed of monosodium phosphate (10 mM), EDTA (0.5 mM) and sodium octyl sulfate (5 mM) in 850 ml distilled H_2O and 150 ml methanol; this solution was adjusted to pH 3. A flow rate of 1 ml/min was used. Brain regions were homogenized in 10 vol of ice-cold $HClO_4$ (0.1N) containing ascorbic acid (0.05 mmol), EDTA (10 mg%) and epinine or DHBA (internal standard) using a ground glass homogenizer (1.5 ml, Radnoti Glass), then centrifuged at 15,000 rpm for 1.5 min. An aliquot (20 μl) of the supernatant was injected into the HPLC system.

Amine and metabolite concentrations were calculated using the ratio of peak areas of compounds of interest to the peak area of the internal standard. These peak-area ratios were compared to those in a standard (calibration) curve which was prepared for each batch of analyses (Fig. 9, 10).

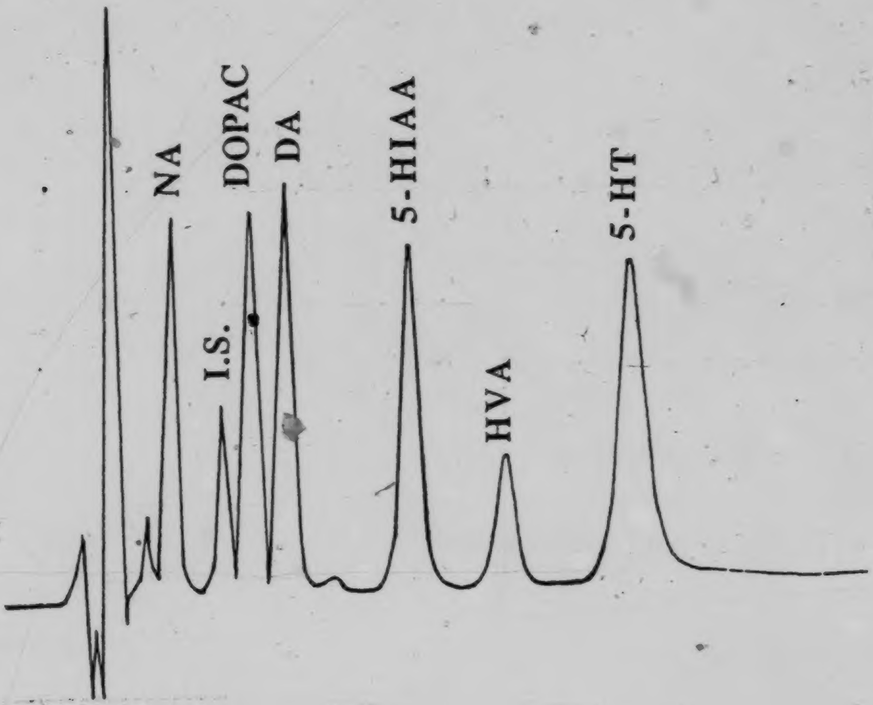


Fig. 9. A typical HPLC run of authentic standards in the analysis of catecholamines, 5-HT and acid metabolites. The concentration of the standards was 50 ng/ml. DHBA (25 ng/ml) was used as an internal standard. The run was complete in 35 min.

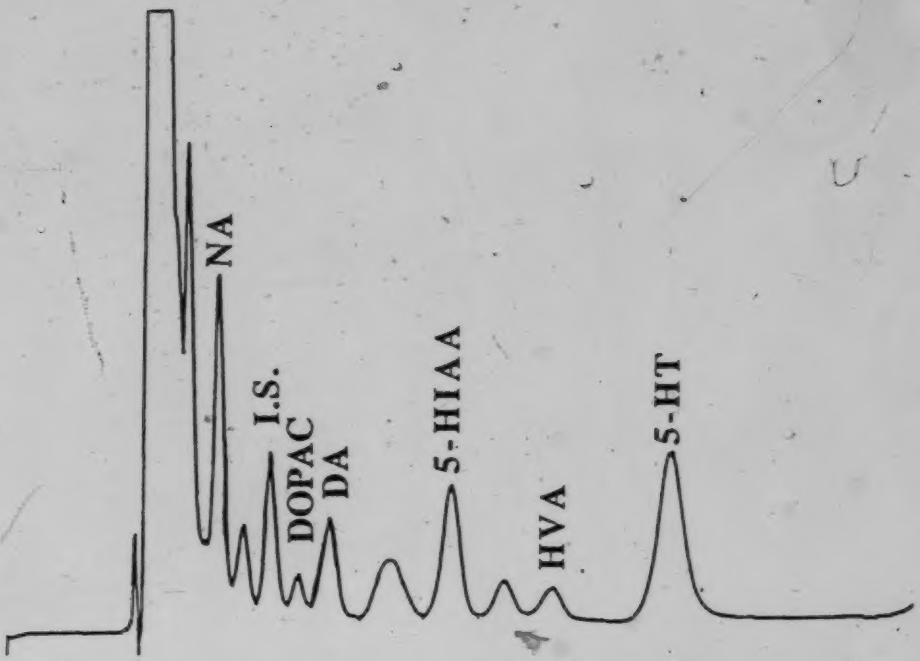


Fig. 10. A typical HPLC run on an extract of cat brain frontal cortex.

2.8.3 Analysis of regional brain levels of GABA

The method used for analysis of the levels of γ-aminobutyric acid (GABA) was based on the procedure developed by Yeung et al. (1986). Brain regions were homogenized in at least 10 volumes (10 ml/g of tissue) of an ice-cold mixture of methanol-6 M aqueous hydrocholoric acid (9:1).

The homogenized brain regions were then placed in microfuge tubes (1.5 ml, Bio Plas) and spun at 5,000 rpm for 1.5 min. A 20 µl portion of the supernatant was used for analysis. α-Methyl-ρ-tyrosine (400 ng) was added as the internal standard. The supernatant was dried under a stream of nitrogen and redissolved in a solution of one drop of concentrated hydrochoric acid in 1 ml of distilled isobutanol. The reaction tube was flushed with nitrogen just prior to capping and heated at 100° C for 30 min. After the reaction tube, containing the isobutanol, was allowed to cool, isobutanol was evaporated under a stream of nitrogen at 50°C. Chloroform (1 ml) containing 5 µl of PFBC solution and 1 ml of saturated aqueous sodium bicarbonate was added to the residue. The samples were shaken for 30 min, spun at 3000 rpm for 1 min and the aqueous layer aspirated off. The organic layer was washed with one ml of distilled water before being evaporated to dryness under a stream of nitrogen. The residue was redissolved in 500 µl decane and a 1 µl aliquot of the decane phase was injected onto a gas chromatograph.

Known amounts of authentic samples of GABA corresponding to 10-1000 ng plus a fixed amount of internal standard, α-methyl-ρ-tyrosine (400 ng), were placed in tubes and carried through in parallel with each set of samples. Standard curves were made by measuring the peak-area ratios of GABA to the internal standard recorded by the GC integrator and plotting this against the known concentrations of GABA. Concentrations of the biological samples were recorded in μmoles/g (Fig. 11, 12).

2.8.4 Monoamine Oxidase Assay

A modification of the method of Wurtman & Axelrod (1963) was used for the measurement of *ex vivo* MAO activity. The caudal one-third of cat cerebrum was homogenized in ice-cold isotonic KCl to give a 1:5 (w/v) homogenate. A portion (25 μl) of the brain tissue homogenate was added to all tubes except blanks, to which only isotonic KCl (25 μl) was added. All tubes were placed on ice and 250 μl of 0.5 M sodium phosphate buffer (pH 7.4) was added to each tube. Suitably diluted solutions of radiolabelled ^{14}C-5-HT (substrate for MAO-A) or ^{14}C-β-phenylethylamine (PEA) (substrate for MAO-B) were added to all tubes (to give final concentrations of PEA and 5-HT of 35 μM). The tubes were then incubated at 37°C for 20 min. After the tubes cooled to room temperature, 2 N HCl (200 μl) was added to each tube to stop the reaction. Toluene (6 ml) was added to all the tubes and the mixtures vortexed for 3 min. The two phases were separated by centrifugation at 2500 rpm for 5 min, and the tubes were then kept in the freezer at -70°C for at least an hour until the aqueous layer was frozen. The toluene

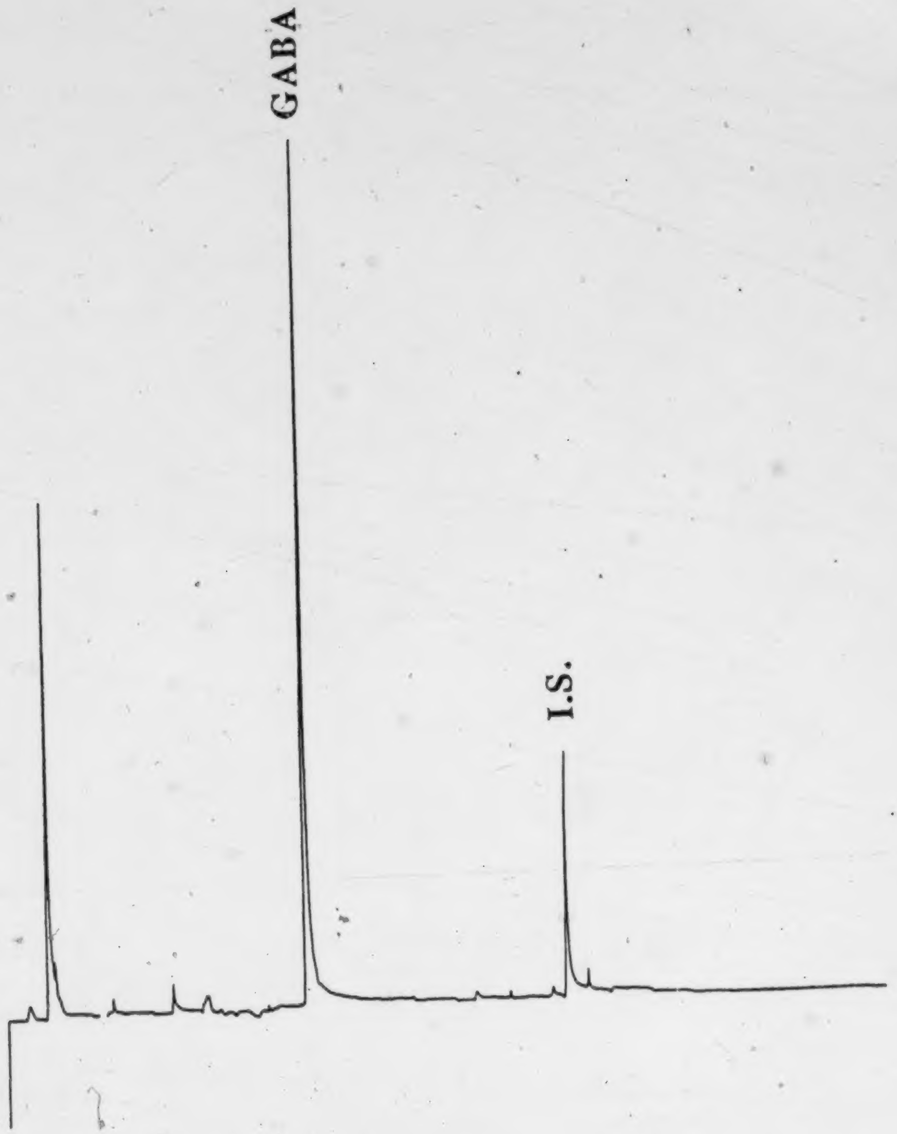


Fig. 11. A typical GC run on authentic standard in the analysis of GABA. The concentration of the standard was 1000 ng. α-Methyl-ρ-tyrosine (400 ng) was used as an internal standard. The run was complete in 22 min.

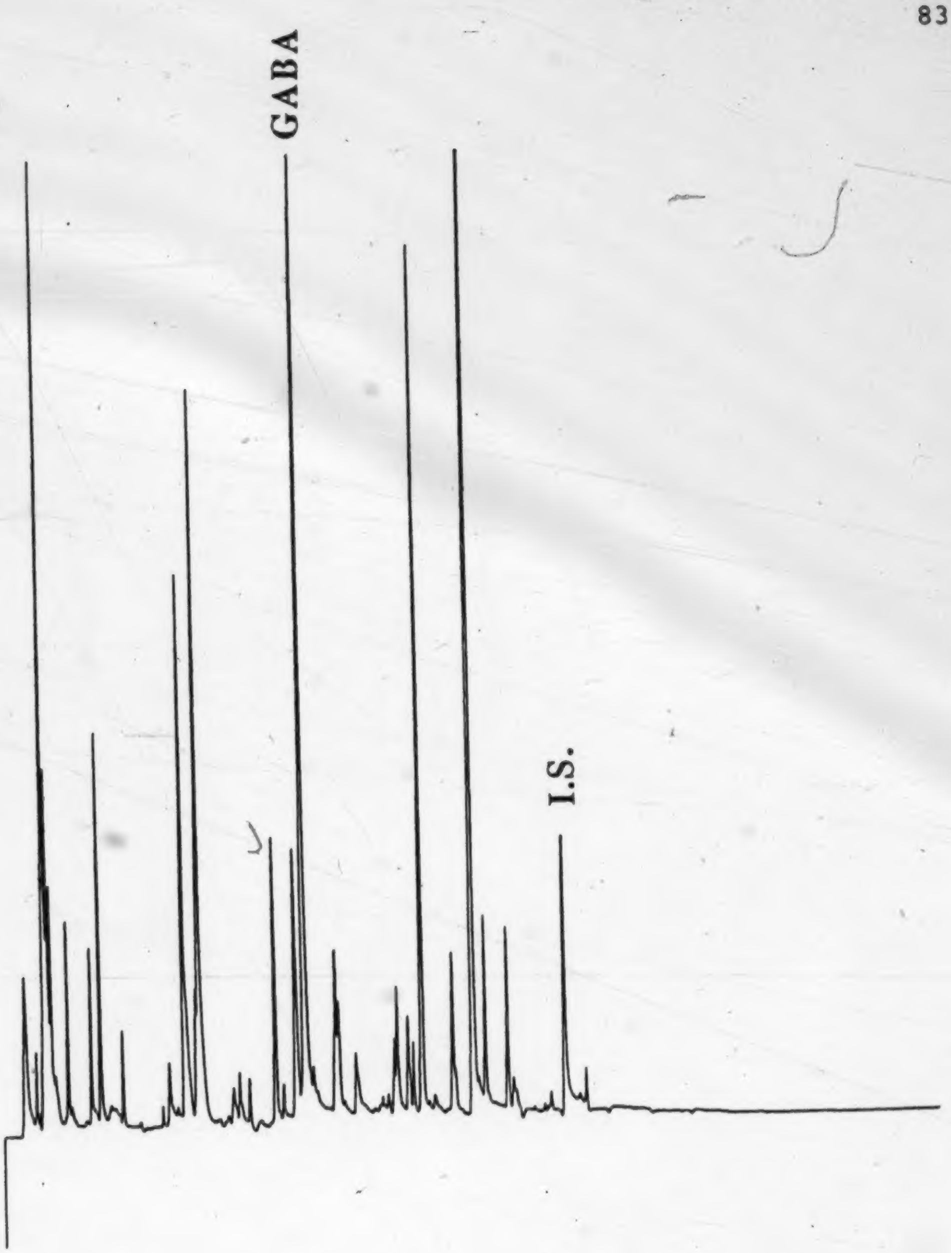


Fig. 12. A typical GC run on an extract of cat brain frontal cortex.

layer was decanted into scintillation vials containing 9 ml of scintillation fluid (4% Butyl-PBD in toluene: Triton X-100, 2:1). The radioactivity (cpm) of each sample was measured in the liquid scintillation spectrometer. The % MAO inhibition was calculated as:

$$\%\ \text{MAO inhibition} = 100 - \left\{ \frac{\text{corrected sample (cpm)}}{\text{mean corrected control (cpm)}} \right\} \times 100$$

Corrected sample and control values were obtained by subtracting the blank values.

2.9 Pathology

Three cats were euthanized with intravenous pentobarbitol upon onset of clinical signs of increased ICP. The brain was immediately removed, sliced coronally, and placed in 10% buffered formalin. After fixation was complete, the slices were embedded in wax and stained with hematoxylin and eosin (H+E), phosphotungstic acidic hematoxylin (PTAH), or glial fibrillary acidic protein (GFAP) or S-100 stain.

2.10 Statistical Analysis

A factorial experiment analysed by 2- or 3-way analysis of variance (ANOVA) permits the separation and evaluation of the effects of two (or more) factors operating in a single experimental design. The factors in this study were a) the presence of the tumor, b) dexamethasone treatment, and c) the brain hemisphere from which the tissue sample was taken. A significant effect, as shown by ANOVA, means that statistical differences exist among the treatment groups. In addition, use of ANOVA permits the detection of interaction effects between two (or more) factors. The term interaction means that factor A may have a different effect when operating in the presence of factor B than when factor B is not present. For example, both the tumor and DEX treatment change neurotransmitter levels; in tumor-bearing brain, levels increase after DEX treatment whereas in control brain the same treatment decreases levels.

Further statistical analysis using 1-way ANOVA followed by the Newman-Keul's test was necessary to determine which particular group of animals had significantly higher or lower values. Sometimes 3-way ANOVA showed that either the tumor, DEX treatment, or the hemisphere from which the sample was taken affected neurotransmitter or water content values, but, the differences were so small that they were not significant between individual treatment groups.

Correlation between white matter water content and tumor diameter was done using correlation coefficient analysis. Tumor diameters in the different groups were compared using 1-way analysis of variance (ANOVA) followed by the Newman Keul's test. Values from untreated tumor-bearing, sham-operated, and control animals were compared using 2-way ANOVA with repeated measures. All values were transformed to the natural log (lnx +1) before analysis. If there was no effect of side, sides were pooled and 1-way ANOVA was done to determine the effect of the tumor. Individuals means were compared post-hoc using the Newman-Keul's test.

Results from tumor-bearing and control animals at the different dosage schedules were compared using 3-way ANOVA to determine the effect of tumor, drug, and hemisphere and any possible interactions. All values were transformed to the natural log (lnx +1) before analysis. Since this required equal numbers in each experimental group, values were randomly removed from some of the groups before analysis was performed. If there was no effect of one or two of the factors, then 2-way ANOVA or 1-way ANOVA was done. Individual means were compared post-hoc using the Newman-Keul's test. Values from vehicle-injected animals were compared to controls using 2-way ANOVA.

The statistical packages used were Statworks and Cricket Graph (Cricket Software, Philadelphia, Pa., U.S.A). The statistical programs for 3-way ANOVA and the Newman-Keul's test were kindly provided by Dr. Andy Greenshaw.

CHAPTER THREE DESCRIPTION OF THE STUDIES

3 The Problem

The presence of a brain tumor is known to regional cerebral blood flow, oxygen consumption, and glucose utilization in adjacent and remote brain tissue, but its effect on brain neurotransmitter levels has received little attention (see sections 1.3.6, 1.3.7). Because brain neurotransmitter levels are disturbed in other brain insults causing brain edema, it is possible that there may be altered neurotransmitter function in peritumor brain tissue (see chapter 1.3.10).

Dexamethasone, a synthetic glucocorticoid, is used in the treatment of various CNS disorders, most notably peritumor brain edema (see chapter 1.6.3). It improves local cerebral glucose utilization in edematous brain tissue (see chapter 1.6.6) and decreases peritumor white matter water content (see chapter 1.6.3). Generally, DEX causes improvement in neurologic function before brain edema is decreased (see chapter 1.6.2). The hypothesis that brain tumors may change peritumor neurotransmitter levels and the possibility that DEX may exert an early beneficial effect on neurologic function through alteration of brain levels of brain neurotransmitter amines, their metabolites, and GABA was explored.

3.1 Study A
Characterization of the Feline Brain Tumor Model

3.1.1 Hypothesis

A larger laboratory animal model for the study of peritumor brain metabolism was needed in order to obtain data for biochemical studies that required several tissue samples from each site. It was proposed that the production of intracerebral 9L glioma tumors in cats would be suitable for these studies.

3.1.2 Objectives

The objectives of this study were to:

1) Determine the success rate and latency of tumor induction using a standardized inoculum of tumor cells.
2) Determine the gross and histologic characteristics of the 9L glioma and surrounding brain.
3) Determine the magnitude and location of brain edema produced by the 9L glioma.

3.1.3 Design

Forty-seven adult mongrel cats were fasted overnight and anesthetized with atropine (0.3 mg/kg), xylazine (2 mg/kg) and ketamine (20 mg/kg) and implanted with 1x10^6 9L glioma cells

(2.2.1-5). Fifteen days later, a group of cats (n=28) was anesthetized and the brains frozen in vivo. These animals did not display abnormal neurologic signs at time of sacrifice. This brain tissue was subsequently used for studies B and C.

On the day that they first exhibited abnormal neurologic signs, another group of cats (n=13) was either euthanized with pentobarbital (n=2) and the brain removed for histologic evaluation, or were anesthetized and the brains frozen in vivo (n=10). One animal died before in vivo freezing could occur. The frozen brain tissue was used for studies B and D. Six animals implanted with 9L glioma cells did not develop any neurologic signs of increased ICP by 3-4 weeks post-implantation. Upon postmortem exam there was no gross visualization of brain tumor. One of these animals was used for histologic studies.

In all animals the brain was cut coronally through the site of tumor injection and the caudal slice of the brain examined for:

a) Presence of a tumor or needle tract.

b) Hemorrhage in or around the tumor.

c) Size and shape of the tumor.

3.2 Study B

Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites, and GABA in Untreated Tumor-Bearing Brain

3.2.1 Hypothesis

Brain tumors cause alterations in cerebral blood flow and metabolism in adjacent and remote brain (see sections 1.3.6, 1.3.7). In other forms of brain insult such as ischemia and triethyltin intoxication there are profound alterations in brain neurotransmitter levels (see sections 1.3.10, 1.3.11). Increases in ICP due to the mass effects of a brain tumor and the associated vasogenic edema may cause brain ischemia. However, it is unknown whether the presence of a brain tumor influences brain neurotransmitters and GABA in the absence of significant increases in ICP.

It was proposed that the presence of a brain tumor in animals without abnormal neurologic signs of increased ICP would be associated with alterations in brain neurotransmitter amines or GABA. The effect of a tumor in animals with abnormal neurologic signs was also assessed.

3.2.2 Objectives

The objectives of this study were to determine whether the presence of a brain tumor altered neurologic status, and/or concentrations of neurotransmitter amines, their metabolites, and GABA in regional brain areas both close to and remote from the tumor in animals without abnormal neurologic signs, and whether the presence of neurologic symptoms indicative of increased ICP had any effect on these changes. In addition, levels of neurotransmitter amines, metabolites, and GABA were measured in the brain tumor itself.

3.2.3 Design

Thirty adult mongrel cats (2.2-6.0 kg) of both sexes were used. There was a control group (n=9), sham-operated group (n=6), and two groups of tumor-bearing animals. Cats (n=9) which did not exhibit any abnormal neurologic signs and cats (n=6) which exhibited anisocoria and somnolence (neurologic signs of increased ICP). Except for control animals, which did not receive an intracranial injection, cats were fasted overnight, anesthetized with atropine (0.3 mg/kg), xylazine (2 mg/kg), and ketamine (20 mg/kg), and implanted with 1 x10^6 9L glioma cells.

In Vivo Freezing of Brain

Fifteen days after xenotransplantation, tumor-bearing and sham-operated animals were examined for neurologic deficits. Clinically normal animals were tranquilized with xylazine (2 mg/kg) and anesthetized with halothane, N_2O and O_2; the head was frozen *in vivo* using a modification of the method of Welsh & Rieder, (1978). The head was removed and stored at -30°C until regions were dissected out; control brains were frozen using the same methods. Tumor-bearing cats with abnormal neurologic signs were sacrificed in an identical fashion on the day that they first exhibited signs.

The head was later thawed to 0°C and dissection of brain regions was done at 0-4°C in a cold room. Sections of brain were analyzed for water content, with adjacent samples taken for neurotransmitter amine and GABA analysis. These samples were stored at -70°C until analysis.

3.3 Study C
Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites and GABA, and Water Content in Tumor-bearing Brain of Cats Without Abnormal Neurologic Signs: Effects of Two Dosages Schedules of Dexamethasone

3.3.1 Hypothesis

Dexamethasone has been used for over 20 years in the treatment of patients with brain tumors. Although one of its main actions is to decrease peritumor brain edema, it has been well demonstrated both clinically and experimentally that neurologic status improves before edema is decreased (see sections 1.6.2, 1.6.3).

In study B the presence of a brain tumor decreased regional monoamine and metabolites and altered GABA levels in animals without abnormal neurologic signs (see chapter 4). Dexamethasone is known to change neurotransmitter levels in normal brain (see sections 1.5.8, 1.5.9); It was postulated that DEX treatment would normalize neurotransmitter amine and GABA levels to control values in tumor-bearing animals that did not exhibit abnormal neurologic signs.

3.3.2 Objectives

In this study, control cats and tumor-bearing cats without neurologic signs of increased ICP were not treated or treated with DEX 1 or DEX 2 dosage schedule. A separate group of control cats were not treated or treated with vehicle at the same schedule as DEX 1 or DEX 2.

The objectives of this study were to determine whether:

1) The administration of DEX at two different dosages altered
    a) neurologic status,
    b) brain neurotransmitter amine, metabolite, or GABA levels,
    c) brain water content

in regional brain areas both close to, and remote from, the tumor in cats without abnormal neurologic signs.

2) The administration of DEX, at two different dosages, altered levels of neurotransmitter amines, their metabolites, and/or GABA and water content in the brain tumor.

3) There was a drug- or vehicle-associated change in neurologic status, brain levels of neurotransmitter amines, their metabolites, and/or GABA, or alterations in brain water content in normal control brain.

3.3.3 Design

Adult mongrel cats were anesthetized with xylazine (2 mg/kg), and ketamine (20 mg/kg). 9L glioma cells (1 x 10^6)

suspended in 50 µl of culture medium were injected 5.5 mm into the central white matter of the brain.

Tumor-bearing and control cats (n=6/group) were given DEX, or left untreated. Separate groups of animals (n=6/group) were not treated or treated with vehicle at the two dosage schedules to determine the effect of the vehicle. Two dosage schedules of DEX were employed; DEX 1 (0.25 mg/kg i.v., 0.25 mg/kg i.m.) with 0.25 mg/kg i.m. repeated in 6 h or DEX 2 (0.25 mg/kg i.v. and 0.25 mg/kg i.m. with 0.25 mg/kg i.m. given 3 more times at 6 h intervals). The head was removed and stored at -30°C until regions were dissected out. All tumor-bearing animals were sacrificed at 15 days post-implantation. For the treated animals sacrifice was timed to occur at 2 h following the last dose of DEX or vehicle. Animals were tranquilized with xylazine (2 mg/kg) and anesthetized with halothane, N_2O, and O_2 and the head was frozen *in vivo*.

The head was later thawed to 0°C and dissection of brain regions was done at 0-4°C in a cold room. Samples were taken for determination of water content, and regional neurotransmitter amine, metabolite, and GABA analysis. The latter were stored at -70°C until analysis.

3.4 Study D
Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites and GABA, and Water Content in Tumor-bearing Brain of Cats with Abnormal Neurologic Signs: Effects of DEX 1 Dosage Schedule

3.4.1 Hypothesis

Treatment with DEX improves neurologic function within a few hours in patients or experimental animals with signs of increased ICP (see sections 1.6.2, 1.6.3). In study B (see chapter 4), neurotransmitter amine and metabolite levels were decreased and GABA increased in the brains of cats with neurologic signs of increased ICP (see chapter 4). Based on observations made in Study C (see chapter 4), where DEX administration tended to normalize neurotransmitter levels, it was hypothesized that the time course of clinical improvement with DEX treatment may be related to changes in brain neurotransmitter levels.

3.4.2 Objectives

In this study, tumor-bearing cats with neurologic signs of increased ICP were not treated or treated with DEX 1 dosage schedule. The objectives of this study were to determine whether there was a DEX-associated change in neurologic status, levels of neurotransmitter amines, metabolites, GABA, or brain water content in brain regions of tumor-bearing cats with abnormal neurologic signs.

3.4.3 Design

Adult mongrel cats were anesthetized with xylazine (2 mg/kg), and ketamine (20 mg/kg). 9L glioma cells (1×10^6) suspended in 50 μl of culture medium were injected 5.5 mm into the central white matter of the brain. Subsequently, some tumor-bearing animals were not treated (n=8) and others were treated (n=5) with DEX 1 (0.25 mg/kg i.v. and 0.25 mg/kg i.m. with 0.25 mg/kg i.m. repeated in 6 h) on the day that they first exhibited abnormal neurologic symptoms. Animals were assessed and scored neurologically before administration of the drug, and every 2 h after until they were anesthetized (6 h later), to determine if there was any effect of the drug on neurologic signs. Animals were tranquilized with xylazine (2 mg/kg) and anesthetized with halothane, N_2O, and O_2 and the head was frozen in vivo. Immediately before in vivo freezing, the intracranial pressure was measured at the level of the cisternae magna. The head was removed and stored at -30°C until regions were dissected out. For the treated animals, sacrifice was timed to occur at 2 h following the last dose of DEX. The head was later thawed to 0°C and dissection of brain regions was done at 0-4°C in a cold room. Samples were taken for determination of water content and regional neurotransmitter amine, metabolite, and GABA analysis. The caudal one-third of the cerebrum was frozen and later used for MAO assay. These samples were stored at -70°C until analysis.

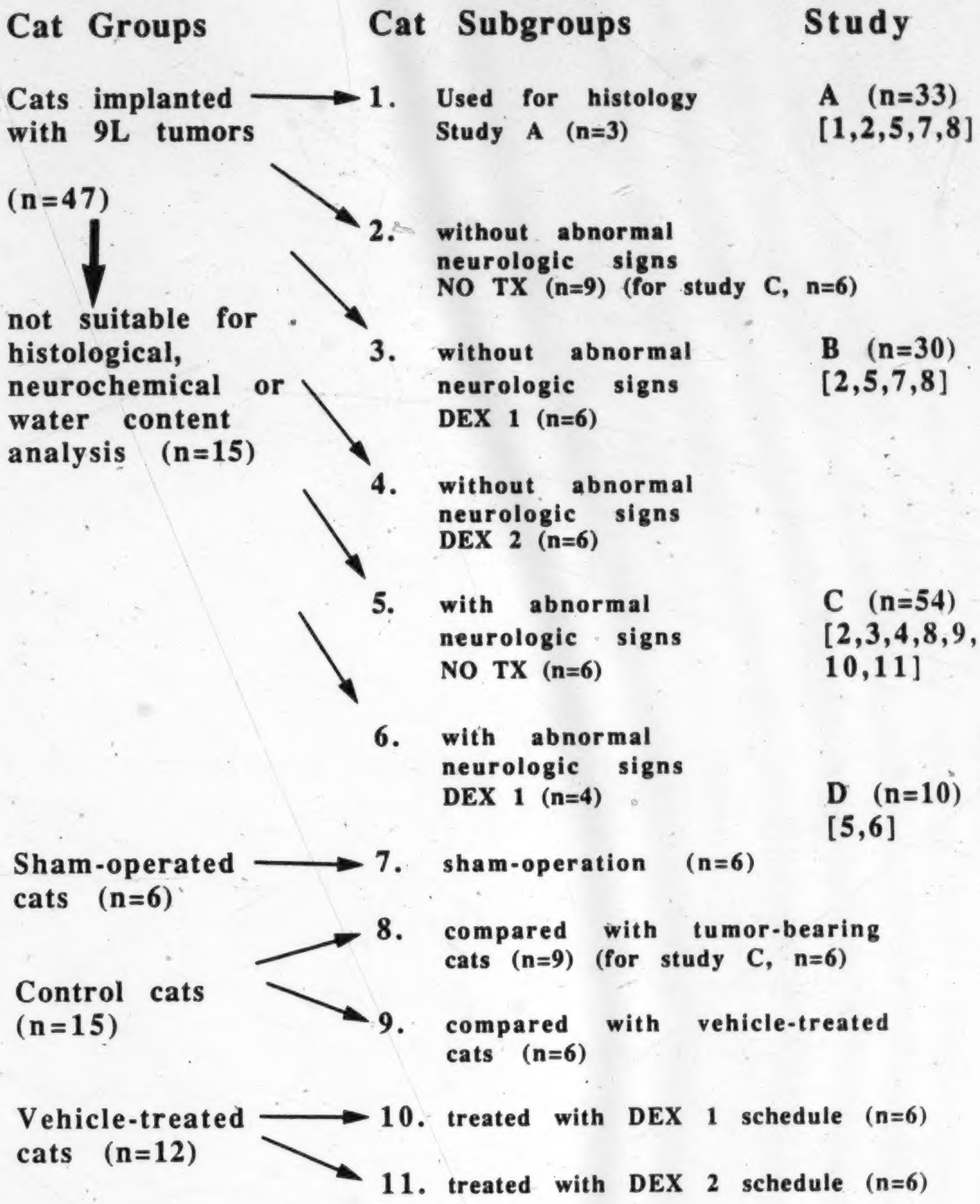

Cat Groups
Cat Subgroups
Study
Cats implanted with 9L tumors
(n=47)
not suitable for histological, neurochemical or water content analysis (n=15)
1. Used for histology Study A (n=3)
2. without abnormal neurologic signs NO TX (n=9) (for study C, n=6)
3. without abnormal neurologic signs DEX 1 (n=6)
4. without abnormal neurologic signs DEX 2 (n=6)
5. with abnormal neurologic signs NO TX (n=6)
6. with abnormal neurologic signs DEX 1 (n=4)
A (n=33) [1,2,5,7,8]
B (n=30) [2,5,7,8]
C (n=54) [2,3,4,8,9, 10,11]
D (n=10) [5,6]
Sham-operated cats (n=6)
7. sham-operation (n=6)
Control cats (n=15)
8. compared with tumor-bearing cats (n=9) (for study C, n=6)
9. compared with vehicle-treated cats (n=6)
Vehicle-treated cats (n=12)
10. treated with DEX 1 schedule (n=6)
11. treated with DEX 2 schedule (n=6)

Fig. 13. Flow chart illustrating the number ( ) and the subgroups [ ] of cats used for histological, neurochemical and water content analysis in each study.

CHAPTER FOUR RESULTS

4.1 Study A

Characteristics of the Feline Brain Tumor Model

4.1.1 Success rate of implantation and incidence of neurologic signs

The tumor implantation success rate was high (75-86%) in both untreated and DEX-treated animals without abnormal neurologic signs. At 15 days post-implantation the tumor size did not differ significantly between groups; however, there was wide variability in the size of the tumor (2-10 mm) in the different cats, with gross hemorrhage in some tumors. In the animals treated with DEX 1 no hemorrhage was observed (Table 3).

Some animals developed signs of increased ICP between 12-21 days post-implantation (Table 4). Untreated cats with abnormal neurologic signs had larger tumors than untreated cats without abnormal neurologic signs [$F(1,14)= 7.88$, $p< 0.05$]. There was no significant difference in incidence of tumor hemorrhage between the the two groups.

Tumor size, percentage of tumors with hemorrhage, and time of onset of clinical signs did not differ between tumor-bearing cats with abnormal neurologic signs treated with DEX and those not treated.

Table 3. Per cent successful induction, per cent of tumors with hemorrhage and diameter of 9L glioma tumors in groups of untreated (NO TX) or dexamethasone-treated (DEX 1, DEX 2) tumor-bearing cats without abnormal neurologic signs.

	% tumor induction	% hemorrhage	tumor diameter (mm) (mean±SEM)
NO TX	11/13 (86%)[†]	5/9 (56%)	5.6 ± 0.9
DEX 1	6/8 (75%)	0/6 (0%)	7.6±1.1
DEX 2	6/7 (86%)	2/6 (33%)	5.4±1.0

[†] Two animals were not suitable for morphological and biochemical studies.

Table 4. Percentage of cats with abnormal neurologic signs, number of days from implantation to onset of clinical signs (days from implantation to abnormal neurologic signs), percentage of tumors with hemorrhage and diameter of 9L gliomas in untreated (N-NO TX) and dexamethasone-treated (N-DEX 1) tumor bearing cats

	N-NO TX	N-DEX 1
% Neurologic Signs	8/12 (68%)†	5/7 (71%)‡
Days from implantation to abnormal neurologic signs (mean±SEM)	15.8 ± 1.2	13.2±1.3
% hemorrhage	5/6 (83%)	2/4 (50%)
tumor diameter (mm) (mean±SEM)	9.2±1.3	8.0±1.0

†Two animals were not suitable for morphological and biochemical studies.

‡One animal was not suitable for morphological and biochemical studies.

4.1.2 Gross Pathology

Upon gross dissection, the dura was thickened and adhered to the site of tumor injection. The parasylvian and marginal gyri were generally swollen and occasionally the tumor was visible on the surface of the cortex. On brain section the tumor appeared as a grey spherical mass centrally located in the centrum ovale but with portions of the tumor present in the cortical regions. The tumor size was quite variable (2-14 mm diameter) (Fig. 14). Hemorrhage around the tumor was observed in approximately half of the animals. Edema in white matter of the affected hemisphere was observed in most brains. Marked mass effect with compression of the contralateral hemisphere was observed in animals with neurologic signs of increased ICP.

4.1.3 Microscopic pathology

Two animals with neurologic signs of increased ICP and one animal which had not shown any signs of tumor growth after three weeks were euthanized and the brains examined histologically. The following is a description of the histologic findings.

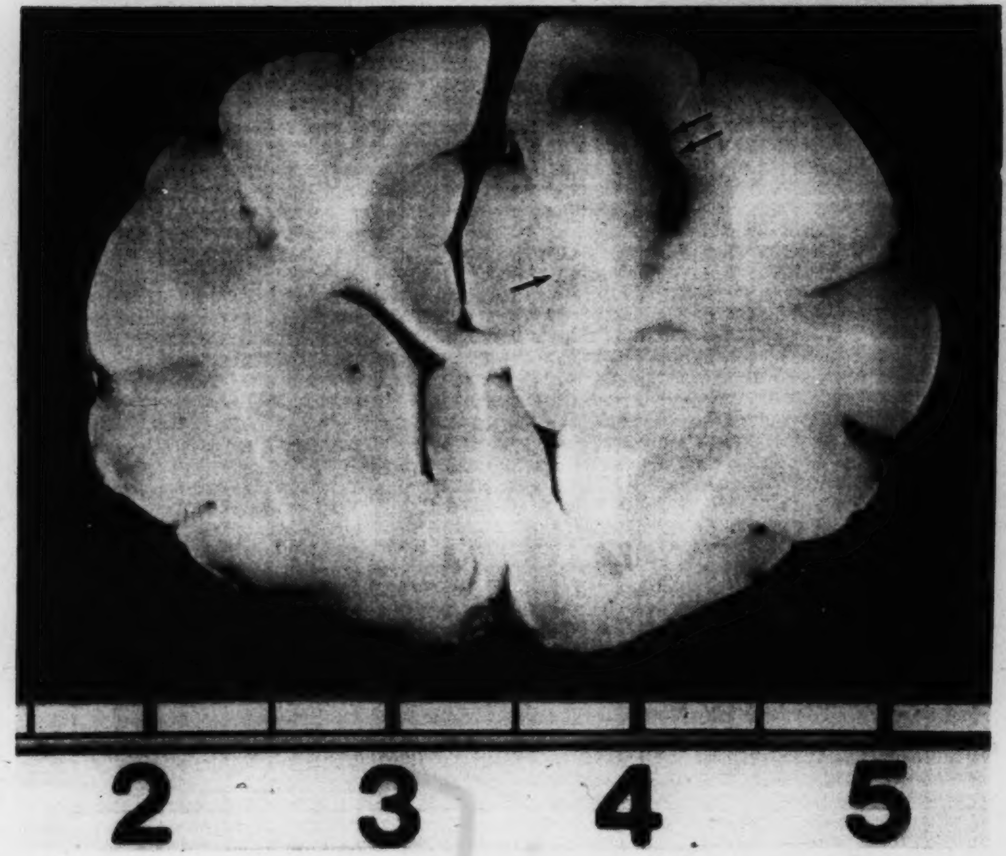


Fig. 14. Coronal view of a 9L glioma (⟶) implanted in the central white matter of a cat brain. Zone of hemorrhage (⇉) around the tumor.

CAT #171-87

Situated within the central white matter of one cerebral hemisphere was a nodular deposit of a poorly differentiated tumor which had focally eroded through the mantle of the adjacent cortex into the subarachnoid space and had grown into the body of the ipsilateral lateral ventricle. The tumor consisted of interlacing, swirling fascicles of elongate spindle cells possessing delicate fibrillated bipolar processes. In a few cells these processes were shown to contain thin PTAH fibrils. As shown in immunohistochemical preparations, none of the tumor cells were reactive for S-100 protein, but a few of these tumor cells showed diffuse, moderately strong immunostaining for GFAP. These reactive tumor cells were dispersed randomly and individually among the population of neoplastic cells and tended to have plumper, more rounded (protoplasmic), cell bodies. The moderately pleomorphic oval nuclei of the tumor cells were disproportionately enlarged, and displayed a course hyperchromasia and occasional small nucleoli. Mitotic figures were common and some were bizarre; multinucleate giant cells were also relatively frequent. In Gomori trichrome preparations no extracellular depositions of collagen or matrix substance were demonstrated within the parenchyma of the tumor. There were no foci of necrosis, but along one margin of the implant there was a zone of fresh hemorrhage. This neoplastic implant was sharply demarcated from the neighbouring neural parenchyma and was circumscribed by a patchy infiltrate of chronic inflammatory cells, which also cuffed many of the nearby blood vessels.

Along the tumor margin there was a mild increase in the blood vessels and scattered widely throughout the white mattter were patchy edematous foci of rarefaction accompanied by mild astrocytosis. These reactive astrocytes displayed strong immunostaining for S-100 protein and, less commonly, GFAP. Several of the blood vessels in the vicinity of the tumor showed prominent endothelial hypertrophy and hyperplasia.

CAT #200-87

Demonstrated in coronal sections of the cerebrum was a multinodular tumor implant within the central white matter of one hemisphere. This implant extended deeply into the anterior limb of the internal capsule and through the overlying cortex into the sulcal subarachnoid space. The poorly differentiated tumor was comprised of whorling, interdigitating fascicles of elongated spindled cells with tapering bipolar processes. Within the fibrillated processes of a few of the neoplastic cells there were demonstrated sparse numbers of fine PTAH fibrils aligned adjacent to the cytoplasmic borders of the cells. In immunohistochemical preparations no immunoreactivity was demonstrated in the tumor cells for S-100 protein and only a very few of the tumor cells displayed the presence of GFAP. In these immunoreactive cells the GFAP immunoreactivity was diffuse but weak within the somas of the stained cells. The disproportionately enlarged, oval nuclei of the neoplastic cells displayed moderate pleomorphism, stippled hyperchromasia and small nucleoli. Mitotic figures were very common as were uninucleated and multinucleated tumor giant cells. Foci of necrosis were present only in the subarachnoid margins of the tumorous deposits and were suffused by infiltrates of acute inflammatory cells. Gomori trichrome preparations failed to demonstrate the presence of extracellular deposits of collagen or matrix material in the tumor parenchyma.

The implant was relatively well demarcated from the surrounding neural parenchyma but along some of the margins there were focal regions in which sheaths of tumor cells had spread along the perivascular spaces of the blood vessels (Fig. 15). The blood vessels along the tumor circumference appeared to be increased in number, displayed prominent endothelial hypertrophy and hyperplasia, and were occasionally cuffed by chronic inflammatory cell infiltrates.

Small foci of edematous rarefaction were scattered within the central and subcortical white matter of the hemisphere and to a lesser extent within the internal capsule. Within the white matter encapsulating the borders of the tumor there were numerous reactive astrocytes which displayed strong immunostaining for GFAP. Similar strong immunoreactivity for S-100 protein was present in the glial cells of cerebral white and grey matter. Some of these glial cells were shown to have become entrapped along the advancing margins of the tumor. There were no lesions present in the contralateral hemisphere.

CAT #46-87

Within the subcortical white matter of the superior medial region of the cerebral hemisphere there was a resorbing tract of necrosis that encroached upon but did not reach the wall of the lateral ventricle. This necrotic tract consisted of a linear core of Gitter cells (foamy macrophages) outlined by a margin of proliferating capillaries and a border of mild reactive astrogliosis. An occasional nearby blood vessel was cuffed by infiltrates of chronic inflammatory cells but there were no deposits of tumor cells present.

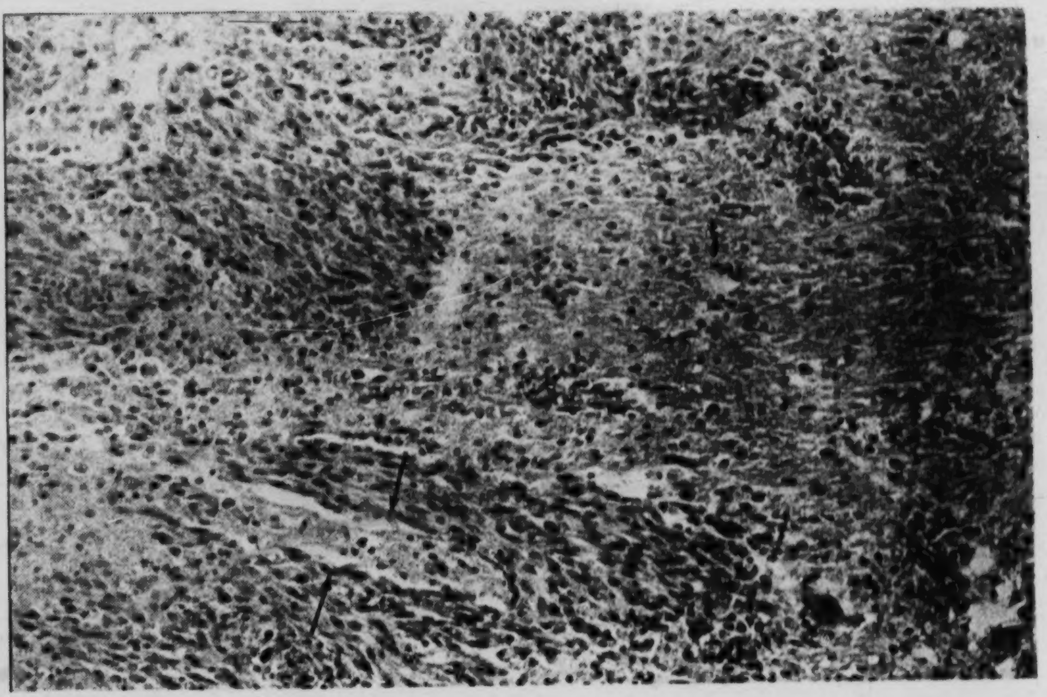


Fig. 15. Histologic photograph illustrating invasion of the tumor (⟶) along the perivascular space into surrounding brain. H & E, 250x

4.1.4 Water content

The per cent water content in tumor tissue in cats without (A) or with abnormal neurologic signs (N) was 85.6 ± 0.8 and 85.5 ± 1.0 (mean ± SEM) respectively. Parietal, temporal, and frontal grey matter values in the tumor-bearing hemisphere were not different from those obtained for corresponding regions in contralateral and control tissue (range = 80.8--82.2%). The white matter adjacent to the tumor had a higher percent water content (73.7 ± 1.7, (A), 75.0 ± 1.6 (N)) than the contralateral side (68.8 ± 0.7, $p< 0.05$; 67.8 ± 0.5, $p< 0.01$), sham-operated (69.4 ± 0.8, $p< 0.05$, $p< 0.01$), and control white matter (67.8 ± 0.6, $p< 0.01$, $p< 0.01$) in equivalent areas. No significant alterations in frontal white matter water content were found.

There was no correlation between tumor size and per cent water content in adjacent parietal white matter in untreated cats without abnormal neurologic signs but there was a negative correlation ($r^2= 0.82$, $p< 0.05$) between tumor size and adjacent parietal white matter water content in untreated cats with abnormal neurologic signs.

4.1.5 Conclusions

This model had a high success rate in both untreated and DEX-treated animals. At 15 days post-implantation most animals had tumors with significant white matter peritumor edema. Some animals developed neurologic signs of increased

ICP at about 2 weeks post-implantation. These animals had larger tumors than animals without abnormal neurologic signs. In both gross and light microscopic examination the experimental glioma resembled a human glioblastoma multiforme.

4.2 Study B

Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites, and GABA in Untreated Tumor-bearing Brain

4.2.1 Neurologic status

Tumor-bearing animals (n=11) without abnormal neurologic signs were sacrificed 15 days post-implantation. Nine animals were suitable for biochemical and water content studies. One animal had the tumor implanted in the wrong location and in the other animal the brain regions were not dissected out correctly. Animals with anisocoria and decreased motor activity (n=8) were sacrificed on the day that they first showed these abnormal neurologic signs. Six animals were suitable for biochemical and water content studies. One animal died suddenly and the other animal was euthanized and the brain used for histological evaluation. Nine control animals and 6 sham-operated animals were sacrificed in an identical fashion. Arterial blood pressure, pH, pCO_2, and pO_2 levels measured immediately prior to sacrifice were not different between the groups.

4.2.2 Intracranial pressure

The intracranial pressure in the animals without abnormal neurologic signs was not measured, but tumor-bearing cats in a similar study had ICP measurements ranging from 2-6 mm Hg (Lazareff, personal communication). Normal control cats anesthetized with the same anesthetic regime had ICP levels ranging from 0-5 mm Hg. Tumor-bearing cats with signs of increased ICP had a mean value of 13.5 ± 0.5 mm Hg immediately prior to sacrifice.

4.2.3 Regional levels of neurotransmitter amines and their metabolites

a) Tumor

The glial cell composition of the 9L tumor is consistent with the low neurotransmitter and metabolite levels seen in this study (Table 5). Levels of 5-HIAA in the tumors of animals with abnormal neurologic signs were higher [$F(1,11)= 11.07$, $p< 0.01$] than in animals without abnormal neurologic signs. For comparison, the monoamine and metabolite levels in cultured 9L glioma cells are also presented.

Table 5. Neurotransmitter amine and metabolite levels (ng/g wet weight) in 9L tumor tissue and 9L glioma cells (mean ± SEM; n=5-8)

	A	N	C
NA	15±6	56±27	61±6
DA	14±8	41±21	67±5
5-HT	81±25	88±27	0±0
DOPAC	14±7	18±13	11±3
HVA	54±26	79±53	0±0
5-HIAA	17±7	141±46**	64±9

A Tumor-bearing animals without abnormal neurologic signs

N Tumor-bearing animals with abnormal neurologic signs

C 9L glioma cells grown in cell culture

**p<0.01 Compared to tumor-bearing animals without abnormal neurologic signs

b) Parietal Grey Matter

The tumor had no effect on the levels of NA (Table 6). Dopamine levels were different between the groups [F(3,56)= 7.49, $p < 0.01$], with the effect being equal in both hemispheres. Tumor-bearing animals ($p < 0.01$) had DA levels 50% lower than seen in control and sham-operated animals. The metabolites DOPAC [F(3,56)=16.42, $p < 0.01$] and HVA [F(3,54)= 3.78, $p < 0.05$] were decreased bilaterally in a similar fashion in both groups of tumor-bearing animals. Sham-operation had no effect on levels of NA, DA, DOPAC, and HVA.

Levels of 5-HT were not affected by the tumor-implantation or sham-operation but 5-HIAA levels were different between groups, with tumor-bearing cats with abnormal neurologic signs having higher levels than control ($p<0.01$), sham-operated ($p< 0.01$), and tumor-bearing cats without abnormal neurologic signs ($p<0.05$).

c) Temporal Grey Matter

Concentrations of NA were different between groups [F(3,51)= 4.80, $p< 0.01$], with both groups of tumor-bearing cats ($p < 0.05$) having lower NA levels than control cats (Table 7). The differences between tumor-bearing and sham-operated groups were not significant. Levels of DA [F(3,51) = 3.79, $p< 0.05$], DOPAC [F(3,49)= 16.66, $p< 0.01$] and HVA [F(3,49)= 4.89, $p< 0.01$] varied between the control, sham-operated, and tumor-

Table 6. Neurotransmitter amine and metabolite levels (ng/g wet weight) in parietal grey matter (mean ± SEM; n=6-9)

	NA	DA	5-HT	DOPAC	HVA	5-HIAA
RC	218±17	107±9	191±30	53±9	114±47	89±13
LC	194±20	112±9	206±33	34±7	88±21	79±12
RSH	220±13	122±26	258±47	35±5	105±8	118±11
LSH	260±25	117±26	221±36	40±5	78±12	117±26
RA	222±38	55±10**	237±50	7±3**	53±21*	82±18
LA	224±32	85±8**	213±43	27±14**	55±21*	78±15
RN	165±17	45±5**	280±33	2±1^{**c}	60±12*	155±28^{*c}
LN	188±27	68±15**	269±43	4±3^{**c}	65±23*	164±35^{*c}

RC: Right hemisphere, control animals
LC: Contralateral hemisphere

RSH: Right hemisphere, sham-operated animals
LSH: Contralateral hemisphere

RA: Right tumor-bearing hemisphere, animals without abnormal neurologic signs
LA: Contralateral hemisphere

RN: Right tumor-bearing hemisphere, animals with abnormal neurologic signs
LN: Contralateral hemisphere

$^{*}p<0.05$ compared with control and sham-operated animals

$^{**}p<0.01$ compared with control and sham-operated animals

$^{c}p<0.05$ compared with animals without abnormal neurologic signs

Table 7. Neurotransmitter amine and metabolite levels (ng/g wet weight) in temporal grey matter (mean ± SEM; n=6-7)

	NA	DA	5-HT	DOPAC	HVA	5-HIAA
RC	434±61	147±25	650±175	98±18	141±41	392±18
LC	426±66	128±18	498±109	74±18	163±50	318±44
RSH	335±26	125±32	448±58	56±10	145±31	247±54
LSH	315±36	98±21	292±21	53±12	147±27	259±35
RA	258±41†	62±19	254±52	62±19	57±11†	193±36
LA	243±25†	100±22	255±50	52±16	116±28†	179±28
RN	248±31†	62±11†	560±130	4±2**	45±11*	327±62
LN	285±29†	73±11†	364±60	6±3**	41±7*	251±53

RC: Right hemisphere, control animals
LC: Contralateral hemisphere

RSH: Right hemisphere, sham-operated animals
LSH: Contralateral hemisphere

RA: Right tumor-bearing hemisphere, animals without abnormal neurologic signs
LA: Contralateral hemisphere

RN: Right tumor-bearing hemisphere, animals with abnormal neurologic signs
LN: Contralateral hemisphere

‡ $p<0.05$ compared with control animals

† $p<0.05$ compared with sham-operated animals

* $p<0.05$ compared with control and sham-operated animals

** $p<0.01$ compared with control and sham-operated animals bearing groups. Only HVA levels were significantly decreased bilaterally in animals without abnormal neurologic signs ($p < 0.05$); in animals with abnormal neurologic signs DA ($p < 0.05$) and its metabolites DOPAC ($p < 0.01$) and HVA ($p < 0.05$) were all decreased bilaterally. Levels of monoamines and metabolites in sham-operated animals were not significantly different from controls.

There was a trend towards a difference in 5-HT levels between groups [$F(3, 51) = 2.57$, $p = 0.07$] but it was not significant. Levels of 5-HIAA were not different between groups.

d) Frontal Grey Matter

There were no differences in neurotransmitter amine and metabolite levels between the control, sham-operated and tumor-bearing groups of cats (Table 8).

e) White Matter

Neurotransmitter amine and metabolite levels were measured in parietal and frontal white matter and found to be highly variable. This variability, which precluded statistical analysis, may have been a reflection of varying amounts of white matter edema.

Table 8. Neurotransmitter amine and metabolite levels (ng/g wet weight) in frontal grey matter (mean ± SEM; n=6-9)

	NA	DA	5-HT	DOPAC	HVA	5-HIAA
RC	364±33	110±15	427±85	71±18	102±22	202±42
LC	367±26	98±13	287±34	78±34	103±24	184±38
RSH	378±44	88±4	307±36	44±11	81±11	177±15
LSH	330±22	83±13	341±48	49±6	70±8	164±11
RA	317±35	101±14	261±46	41±7	64±11	141±23
LA	315±54	90±11	214±51	51±10	119±28	112±13
RN	298±20	110±13	228±35	51±3	128±26	197±16
LN	387±48	148±35	368±65	60±13	134±45	250±55

RC:	Right hemisphere, control animals
LC:	Contralateral hemisphere
RSH:	Right hemisphere, sham-operated animals
LSH:	Contralateral hemisphere
RA:	Right tumor-bearing hemisphere, animals without abnormal neurologic signs
LA:	Contralateral hemisphere
RN:	Right tumor-bearing hemisphere, animals with abnormal neurologic signs
LN:	Contralateral hemisphere

4.2.4 Regional GABA levels

a) Tumor

GABA levels in the brain tumor were low compared to surrounding grey matter. In animals with neurologic signs tumor levels were higher (1.89 ± 0.29 µmoles/g) than in animals without neurologic signs (0.78 ± 0.29 µmoles/g) [F(1,10)= 6.44, $p < 0.05$]. GABA levels in cultured 9L glioma cells were also very low (0.13 µmoles/g).

b) Grey matter

In grey matter adjacent to the tumor (parietal), GABA levels differed between groups [F(3,52)= 8.93, $p < 0.01$], with tumor-bearing animals with abnormal neurologic signs having higher GABA levels ($p < 0.01$) than control, sham-operated, and tumor-bearing animals without abnormal neurologic signs (Table 9). There were no differences between hemispheres or group-hemisphere interactions. In grey matter somewhat further from the tumor (temporal) there were no significant differences in GABA levels between the groups. In grey matter remote from the tumor (frontal) there were bilateral changes in GABA levels [F(3,55)= 20.63, $p < 0.01$]. In this area, tumor-bearing animals with abnormal neurologic signs had higher GABA levels ($p < 0.01$) than control, sham-operated, or tumor-bearing animals without abnormal neurologic signs.

Table 9. GABA levels (μmoles/g wet weight) in parietal grey (PG), temporal grey (TG), frontal grey (FG), parietal white (PW) and frontal white (FW) matter. (mean ± SEM, n = 4-9)

	PG	TG	FG	PW	FW
RC	3.03±.34	2.92±.24	2.59±.34	2.56±.39	1.85±.27
LC	2.28±.17	2.80±.22	1.91±.14	2.24±.19	1.85±.20
RSH	2.32±.34	3.77±.15	2.15±.16	1.96±.45	1.51±.12
LSH	2.44±.40	3.33±.31	2.04±.13	2.12±.61	1.72±.16
RA	2.59±.26	3.63±.38	2.54±.11	1.51±.35 ‡	1.23±.30
LA	2.61±.27	3.80±.61	2.62±.31	1.35±.17 ‡	1.38±.32
RN	4.51±.54**	3.23±.26	6.04±.72**	2.25±.42†	1.71±.40
LN	4.02±.49**	3.10±.35	4.43±.65**	1.82±.14†	2.31±.34

RC: Right hemisphere, control animals
LC: Contralateral hemisphere

RSH: Right hemisphere, sham-operation
LSH: Contralateral hemisphere

RA: Right tumor-bearing hemisphere, animals without abnormal neurologic signs
LA: Contralateral hemisphere

RN: Right tumor-bearing hemisphere, animals with abnormal neurologic signs
LN: Contralateral hemisphere

** $p<0.01$ compared with control, sham-operated and tumor-bearing animals without abnormal neurologic signs

† $p<0.05$ compared with animals without abnormal neurologic signs

‡ $p<0.01$ compared with control and sham-operated animals

c) White matter

Levels of GABA in parietal white matter differed between groups [F(3,49)= 5.33, $p < 0.01$]. There was no effect of hemisphere or group-hemisphere interactions. Levels in parietal white matter of tumor-bearing animals without abnormal neurologic signs were lower than those with abnormal neurologic signs ($p < 0.05$), sham-operated ($p < 0.01$) or control animals ($p < 0.01$) (Table 9). There was no effect of group or hemisphere on GABA levels in frontal white matter.

4.2.6 Conclusions

Brain tumors caused significant bilateral decreases in monoamine and metabolite levels in parietal and temporal brain regions; changes were accentuated in tumor-bearing cats with neurologic signs of increased ICP. Sham-operation had no effect on monoamine and metabolite levels. Levels of GABA were decreased in parietal white matter of cats without abnormal neurologic signs, whereas cats with abnormal neurologic signs had increased GABA levels in the tumor and in parietal and frontal grey matter. Levels of GABA were not changed by sham-operation.

4.3 Study C

Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites and GABA, and Water Content in Tumor-bearing Brain of Cats without Abnormal Neurologic Signs: Effects of Two Dosage Schedules of Dexamethasone

4.3.1 Neurologic Status

All tumor-bearing cats were neurologically normal when sacrificed 15 days post-implantation. There were 6 tumor-bearing cats and 6 control cats in each drug treatment group. Separate groups of control cats and cats treated with vehicle at the same dosage schedule as DEX 1 and DEX 2 were used to determine the effect of the vehicle (n=6/group). Control and vehicle-treated animals were sacrificed in an identical fashion to tumor-bearing animals. Arterial blood pressure and pCO_2 measured immediately prior to sacrifice were not different between groups, but there were differences in mean pH [$F(2,38)= 5.33$, $p< 0.01$] (range 7.30-7.37) and pO_2 [$F(2,41)= 9.85$, $p< 0.01$] (range 141- 233 mm Hg), although all values were within normal limits. There were no significant differences in blood pressure, pH, pCO_2, and pO_2 between tumor-bearing and control animals within these groups or between control and vehicle-treated animals.

4.3.2 Intracranial pressure

The ICP was not measured in any of these groups.

4.3.3 Regional levels of neurotransmitter amines and their metabolites

a) Tumor

Levels of NA varied in tumor tissues from different groups [F(2,17)= 14.43, $p < 0.01$], with levels in the DEX 2 group being undetectable (Fig. 16). Dopamine and 5-HT levels were not significantly different. Levels of DOPAC differed between groups [F(2,17)= 6.73, $p < 0.01$], with levels being higher in the DEX 2 group compared to the untreated (NO TX) ($p < 0.01$) or DEX 1 ($p < 0.05$) groups. Levels of HVA remained unchanged. Concentrations of 5-HIAA were altered [F(2,17)= 17.87, $p < 0.01$], with levels in the DEX 1 and DEX 2 groups being significantly higher then in the NO TX ($p < 0.01$) group.

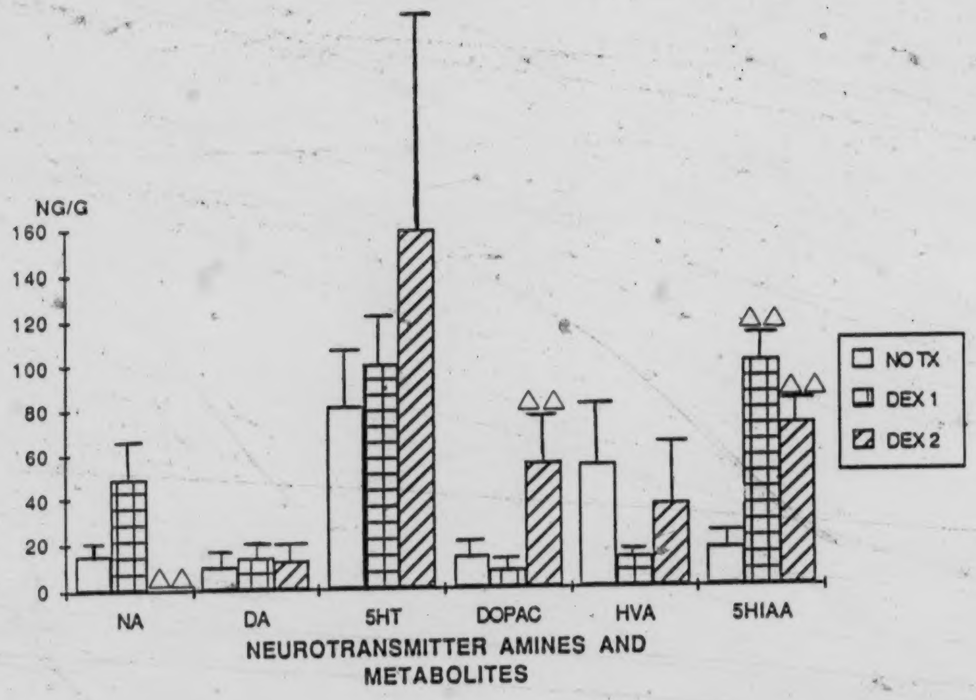


Fig. 16. Levels of NA, DA, 5-HT, DOPAC, HVA, and 5-HIAA (ng/g wet weight ± SEM) in the tumors of animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. (n = 6-9/group)

Effect of DEX
ΔΔ $p < 0.01$ compared with untreated animals

b) Parietal Grey Matter

The presence of the tumor led to significant changes in NA levels [F(1,60)= 4.50, $p < 0.05$] (Fig. 17). There was a small overall DEX effect (i.e., statistical differences existed among the treatment groups) [F(2,60)= 3.47, $p < 0.05$], but this was not significant within either the tumor-bearing or control groups. Levels of NA differed between hemispheres [F(1,60)= 4.05, $p < 0.05$]. The tumor induced a small but significant decrease in NA levels in peritumor grey matter compared to the contralateral hemisphere [F(1,35)= 5.37, $p < 0.05$] which was especially apparent in DEX-treated animals; there was no effect of hemisphere in control brain. Levels of NA were not different between vehicle-injected and control animals

The tumor also affected levels of DA [F(1,60)= 6.79, $p < 0.05$] (Fig. 17). Levels in brain hemispheres differed [F(1,60)= 4.59, $p < 0.05$], although individual comparisons did not reveal any specific differences within treatment groups. Dexamethasone treatment did not have any effect on DA levels; however, there was a tumor-DEX interaction (i.e., DEX treatment altered neurotransmitter levels differently in tumor-bearing animals than in control animals) [F(2,60)= 10.05, $p < 0.01$]. Levels of DA were comparable in vehicle-injected and control animals.

A bilateral decrease in DA levels was seen in untreated tumor-bearing animals ($p < 0.01$) and was still apparent in tumor-bearing animals treated with DEX 1 ($p < 0.05$). In contrast,

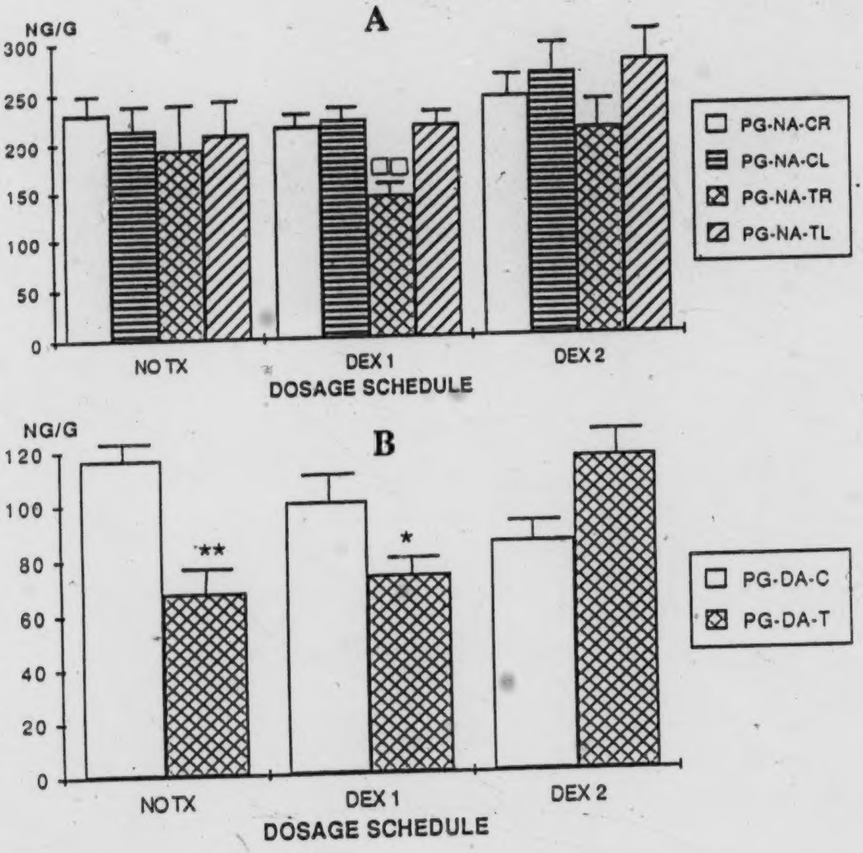


Fig. 17. Levels of NA (A) and DA (B) (ng/g wet weight ± SEM) in the the tumor-bearing (R) and contralateral hemisphere (L) in parietal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels of DA are pooled from both hemispheres. (n=6/group)

Effect of tumor in each treatment group
* $p < 0.05$
** $p < 0.01$

Effect of tumor-bearing hemisphere in each treatment group
$p < 0.01$

A bilateral decrease in DA levels was seen in untreated tumor-bearing animals ($p< 0.01$) and was still apparent in tumor-bearing animals treated with DEX 1 ($p< 0.05$). In contrast, after DEX 2 DA levels were similar to drug-treated controls. The drug itself did not affect control levels of DA (Fig. 16).

The DA metabolite DOPAC, was also affected by the tumor [$F(1,60)= 6.36$, $p< 0.05$] and DEX treatments [$F(2,60)= 27.62$, $p< 0.01$], with an interaction between tumor and DEX [$F(2,60)= 10.33$, $p< 0.01$]. There was no difference between DOPAC levels in control and vehicle-injected animals.

Levels of DOPAC were decreased bilaterally in untreated tumor-bearing animals ($p< 0.01$) (Fig. 18). After a short course of DEX (DEX 1) there was no difference between control and tumor-bearing animals. In animals treated with a longer course of DEX (DEX 2), DOPAC levels were elevated over drug-treated control brain ($p< 0.05$). Multiple comparisons revealed that the drug itself (DEX 1) also caused decreases in DOPAC ($p< 0.01$), although levels returned to normal in control animals treated with DEX 2 (Fig. 18).

Levels of HVA were not affected by the tumor (Fig. 18). Analysis of variance revealed a significant effect of DEX [$F(2,60)= 3.95$, $p< 0.01$]; post-hoc comparisons showed that HVA levels in control and tumor-bearing animals treated with DEX 2 were elevated over untreated and DEX 1-treated animals ($p< 0.01$). Levels of HVA were not different in vehicle-injected compared to control animals.

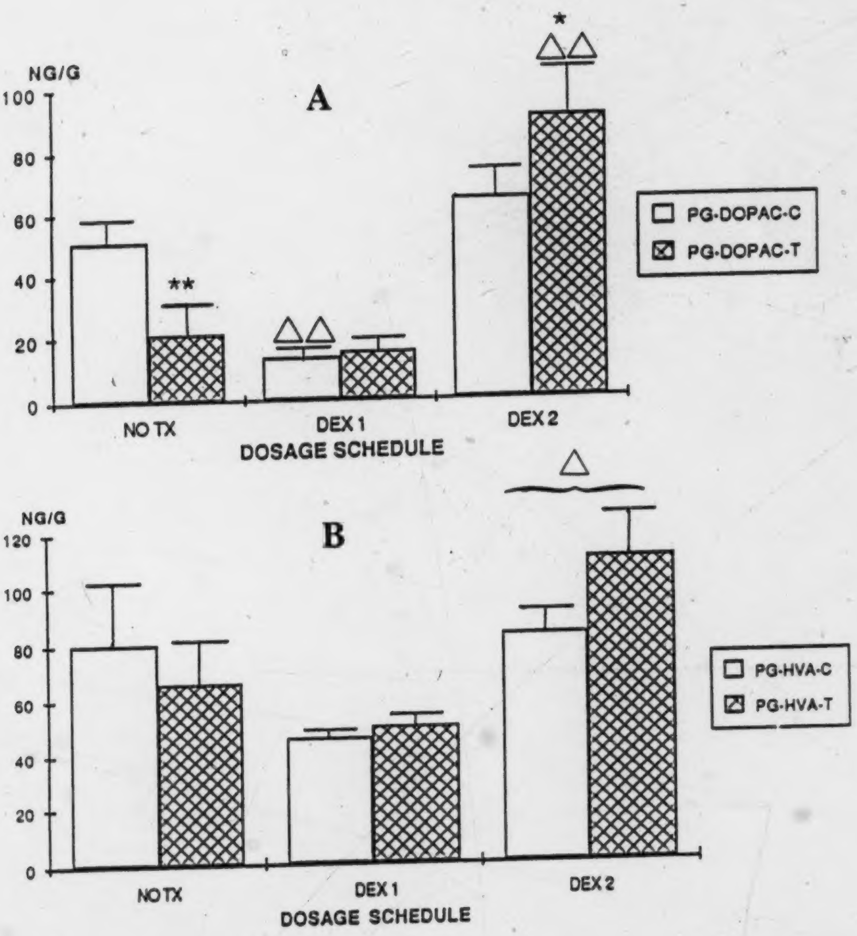


Fig. 18. Levels of DOPAC (A) and HVA (B) (ng/g wet weight ± SEM) in the parietal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n=6/group)

Effect of tumor in each treatment group
* $p < 0.05$
** $p < 0.01$

Effect of DEX in control animals
ΔΔ $p < 0.01$ compared with untreated animals

Effect of DEX in tumor-bearing animals
ΔΔ $p < 0.01$ compared with untreated animals

Effect of DEX in control and tumor-bearing animals
Δ $p < 0.05$ compared with untreated animals

Levels of 5-HT were not affected by either the tumor, DEX, or hemisphere, and there were no significant interactions (Fig. 19). Concentrations of 5-HT were not different in vehicle-injected or control animals. The levels of 5-HIAA were not affected by the tumor, but there was a significant effect of DEX [F(2,60)= 9.58, $p < 0.01$] (Fig. 19). Newman-Keul's tests showed that 5-HIAA levels were increased ($p < 0.01$) in tumor-bearing and control brains after DEX 2 treatment. There were no differences in 5-HIAA levels between vehicle-injected and control animals.

c) Temporal Grey Matter

There was a significant depressant effect of the tumor on NA levels [F(1,48)= 5.33, $p < 0.05$], although this was not significant in any of the separate drug-treated groups (Fig. 20). Treatment with DEX did not have any effect on NA levels and there were no tumor-DEX interactions.

Dopamine levels in the temporal grey matter were not affected by the tumor (Fig. 20). The metabolites of DA (i.e., DOPAC and HVA) were also not affected by either the tumor or DEX (Fig. 21). Animals injected with either dosage schedule of vehicle did not have significantly different levels of catecholamines and metabolites from control animals.

DEX had a significant effect of 5-HT levels in this brain region [F(2,48)= 5.54, $p < 0.01$], with both control and tumor-bearing animals treated with DEX 2 having lower 5-HT levels ($p < 0.05$) than untreated animals or animals treated with DEX 1 (Fig. 22).

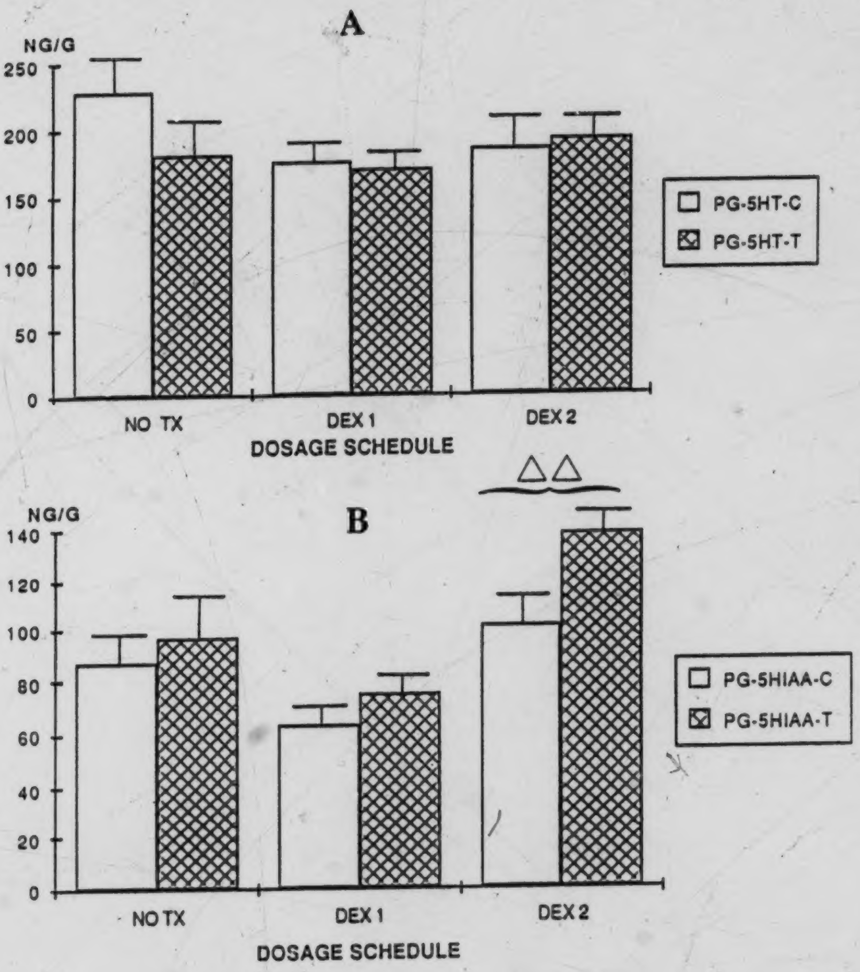


Fig. 19. Levels of 5-HT (A) and 5-HIAA (B) (ng/g wet weight ± SEM) in the parietal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n=6/group)

Effect of DEX in control and tumor-bearing animals
ΔΔ $p < 0.01$ compared with untreated animals

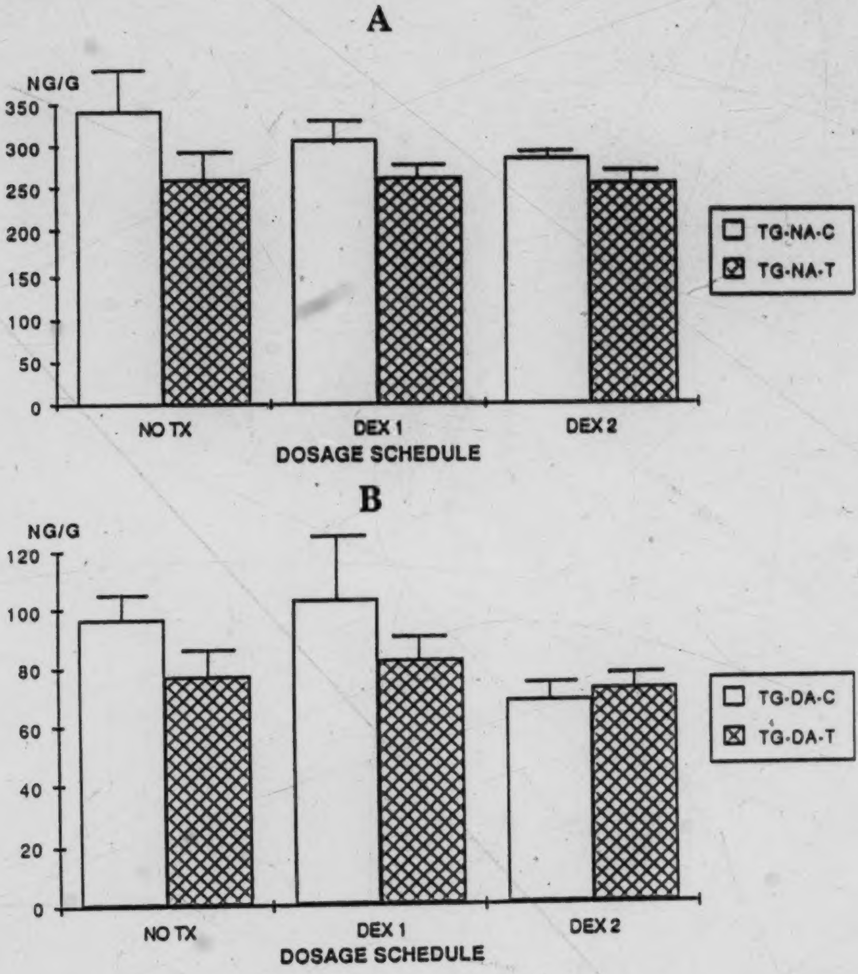


Fig. 20. Levels of NA (A) and DA (B) (ng/g wet weight ± SEM) in the temporal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n=6/group)

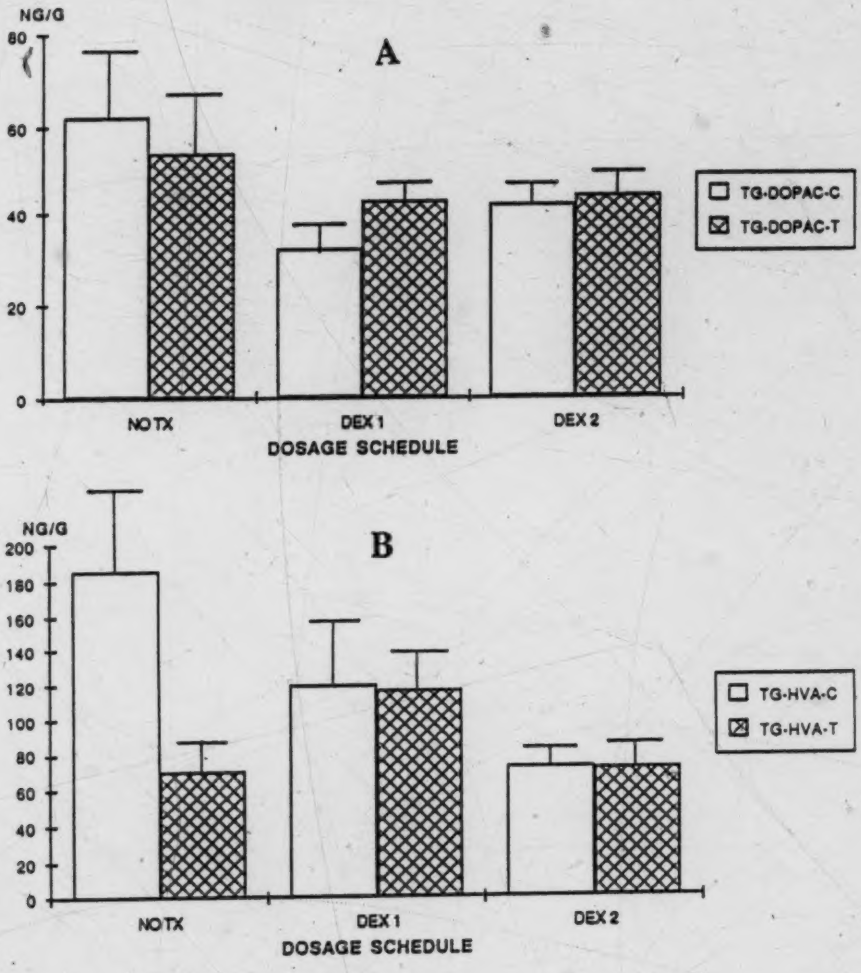


Fig. 21. Levels of DOPAC (A) and HVA (B) (ng/g wet weight ± SEM) in the temporal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n=6/group)

The levels of the 5-HT metabolite 5-HIAA were not altered by either the tumor or DEX (Fig. 21). Vehicle-injection had no significant effect on levels of 5-HT and 5-HIAA in this area.

d) Frontal Grey Matter

There was an effect of the tumor [$F(1,60)= 15.82$, $p< 0.01$] and DEX [$F(2,60)= 4.36$, $p< 0.05$] but not of hemisphere on NA levels. A tumor-DEX interaction [$F(2,60)= 6.76$, $p< 0.01$] was also seen (Fig. 23).

Levels of NA were significantly decreased in DEX 1-treated tumor-bearing animals ($p< 0.01$) compared to untreated and DEX 2-treated tumor-bearing animals. In this group NA levels were also lower ($p< 0.01$) than DEX 1-treated control animals. DEX treatment did not change NA levels in control tissue.

Overall, the tumor decreased DA levels [$F(1,60)=5.37$, $p< 0.05$], although this was not significant within any of the treatment groups (Fig. 23). Administration of DEX also caused changes in DA levels [$F(2,60)= 5.52$, $p< 0.01$]. Control and tumor-bearing animals in the DEX 2 group had lower DA levels ($p< 0.05$) than those in the NO TX group. Levels of the DA metabolite DOPAC were not affected by the tumor, hemisphere or DEX treatment (Fig. 24) The administration of DEX changed HVA levels [$F(2,60)=4.31$, $p< 0.05$] but the tumor did not significantly affect HVA levels [$F(1,60)=3.49$, $p= 0.10$] (Fig. 24). In control brain DEX 1 levels of HVA were lower ($p< 0.05$) than either NO TX or DEX 2 levels. Levels of HVA in tumor-bearing brain were lower in

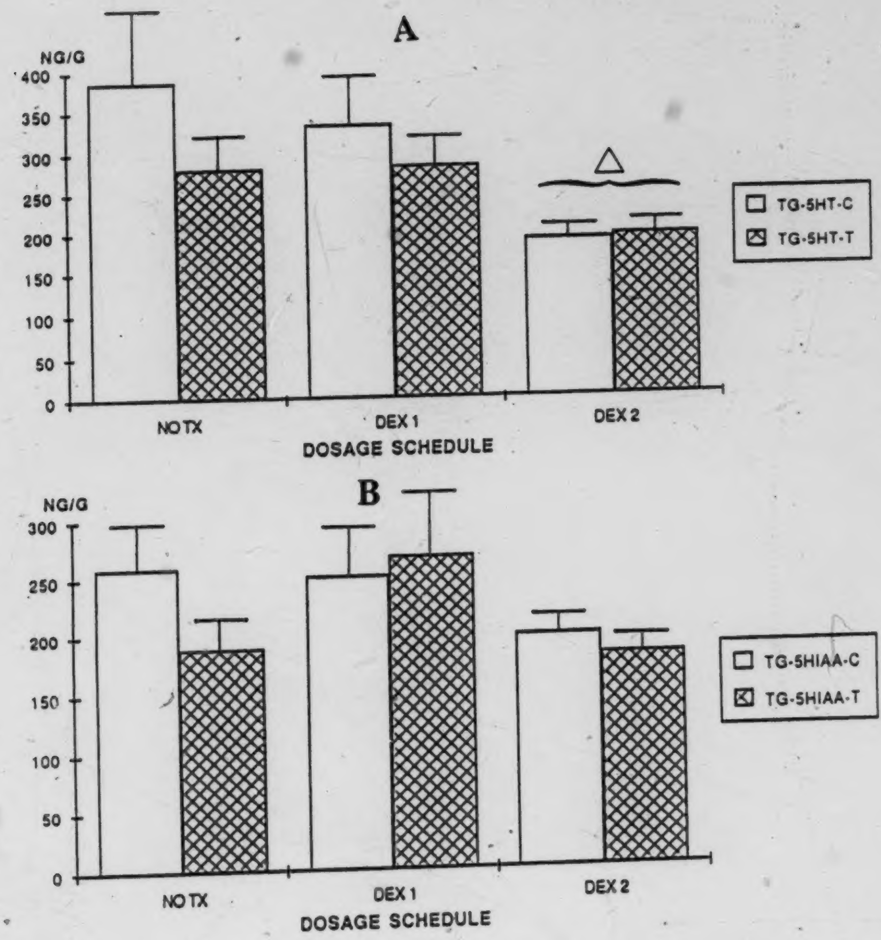


Fig. 22. Levels of 5-HT (A) and 5-HIAA (B) (ng/g wet weight ± SEM) in the temporal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n=6/group)

Effect of DEX in control and tumor-bearing animals
Δ $p < 0.05$ compared with untreated animals

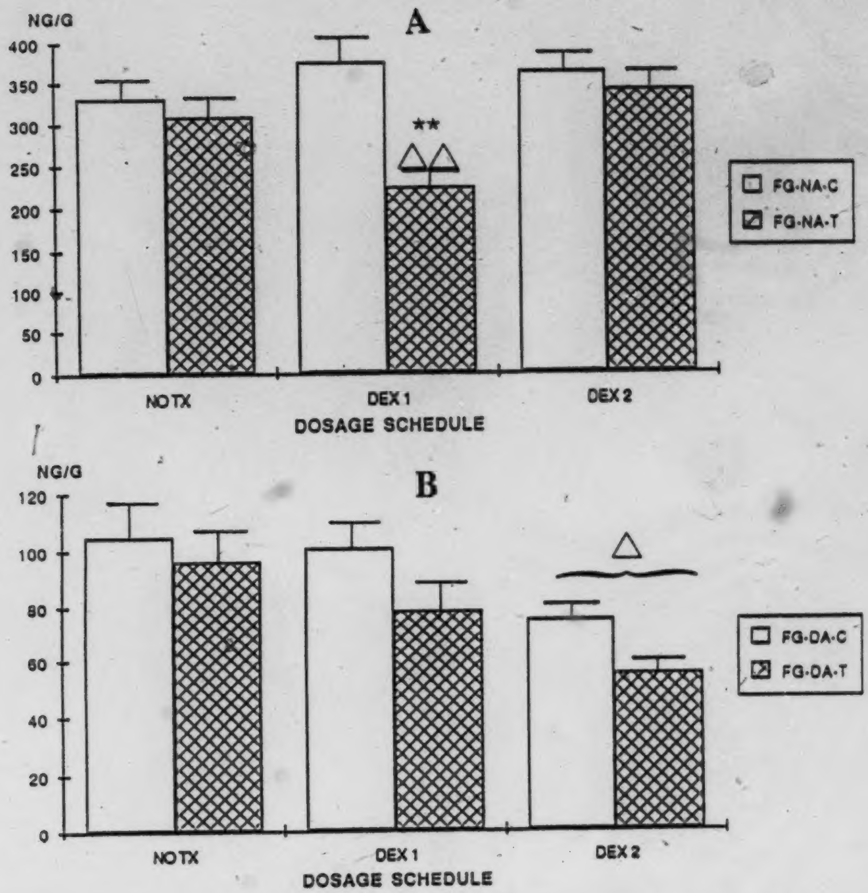


Fig. 23. Levels of NA (A) and DA (B) (ng/g wet weight ± SEM) in the frontal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n=6/group)

Effect of tumor in each treatment group
** $p < 0.01$

Effect of DEX in tumor-bearing animals
$\Delta\Delta$ $p < 0.01$ compared with untreated animals

Effect of DEX in control and tumor-bearing animals
Δ $p < 0.05$ compared with untreated animals

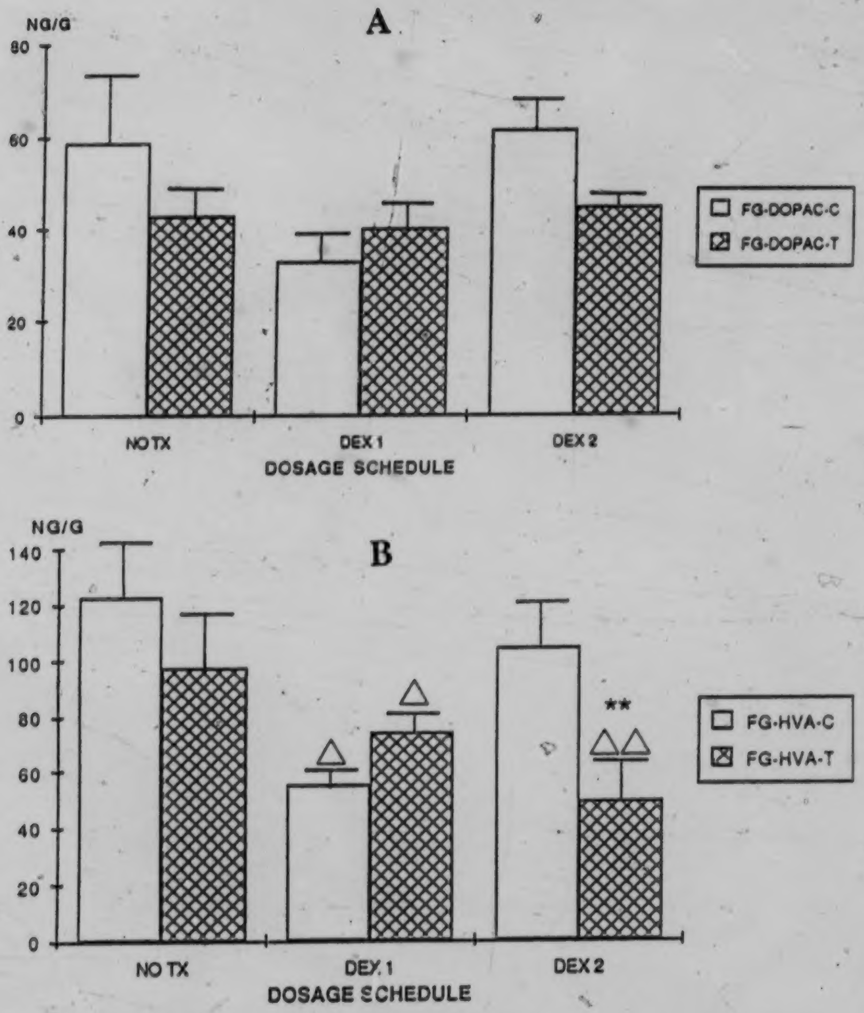


Fig. 24. Levels of DOPAC (A) and HVA (B) (ng/g wet weight ± SEM) in the frontal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n = 6/group)

Effect of tumor in each treatment group
** $p < 0.01$

Effect of DEX in control animals
Δ $p < 0.01$ compared with untreated animals

Effect of DEX in tumor-bearing animals
Δ $p < 0.01$ compared with untreated animals
$\Delta\Delta$ $p < 0.01$ compared with untreated animals

both DEX 1- ($p < 0.05$) and DEX 2- ($p < 0.01$) treated animals compared to untreated tumor-bearing cats. There was an interaction between DEX treatment and tumor [F(2,60)= 6.24, $p < 0.01$], with tumor-bearing animals in the DEX 2 group having significantly lower HVA levels ($p < 0.01$) than drug-treated controls. There was no effect of vehicle injection on NA, DA, DOPAC, or HVA levels.

The tumor affected 5-HT levels [F(1,60)= 4.05, $p < 0.05$], although multiple comparisons did not reveal any significant differences within treatment groups (Fig. 25). The drug also changed 5-HT levels [F(2,60)= 7.64, $p < 0.01$], with animals treated with DEX 2 having lower ($p < 0.01$) 5-HT levels then untreated or DEX 1-treated animals. There was no significant interaction between tumor and DEX treatment.

Levels of 5-HIAA were not specifically altered by either the tumor or DEX treatment but there was a tumor-DEX interaction [F(2,60)= 5.02, $p < 0.01$] in that 5-HIAA levels were higher ($p < 0.01$) in tumor-bearing animals in the DEX 2 group compared to drug-treated controls (Fig. 25).

There were no significant differences in 5-HT and 5-HIAA levels between vehicle-injected and control cats.

e) White Matter

Comparisons between monoamine and metabolite levels in the different groups could not be done because of extreme variability within the groups. This could be a reflection of

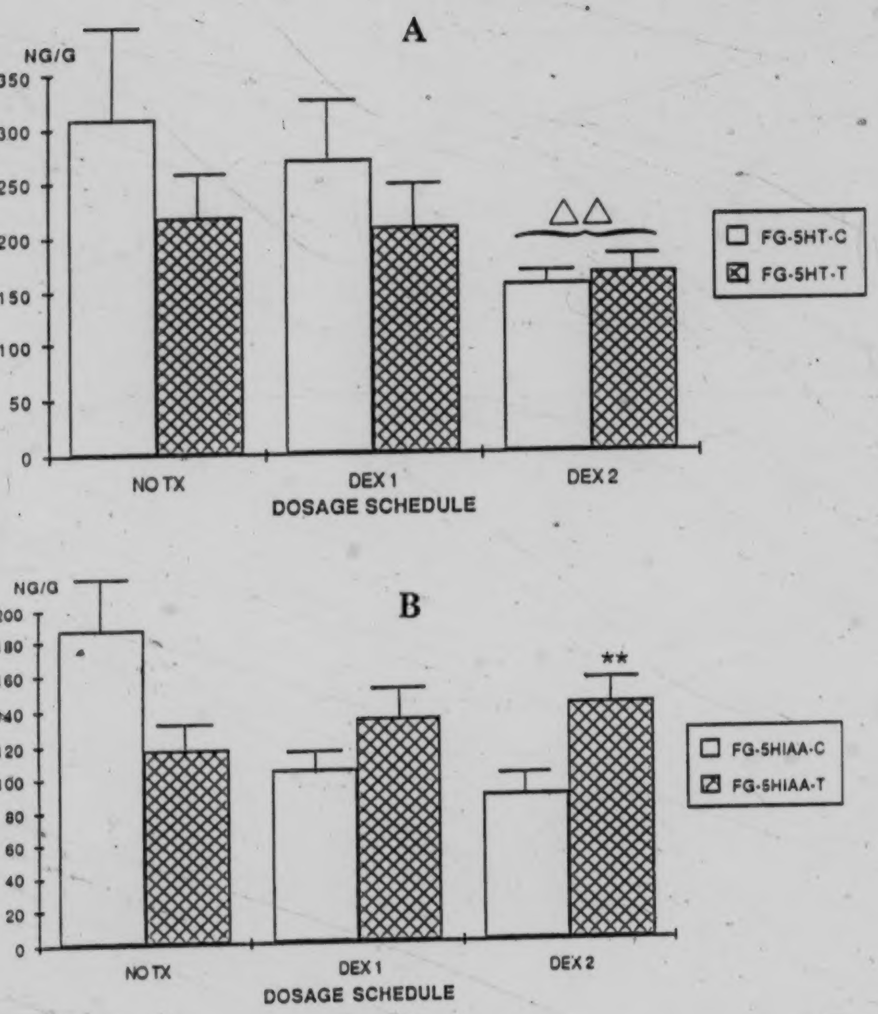


Fig. 25. Levels of 5-HT and 5-HIAA (ng/g wet weight ± SEM) in the frontal grey matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n = 6/group)

Effect of tumor in each treatment group
** $p < 0.01$

Effect of DEX in control and tumor-bearing animals
ΔΔ $p < 0.01$ compared with untreated animals

differing degrees of white matter edema or could be due to other factors.

f) Summary of neurotransmitter amine and metabolite alterations

For an overview, the following tables (Tables 10-15) have been provided in order to illustrate how the tumor, DEX treatment or the interaction between these factors increase, decrease or have no effect on regional neurotransmitter amine or metabolite levels.

4.4.4 Regional GABA levels

Because of 3-way ANOVA requires equal numbers of values in each group (see section 2.10) and because some treatment groups only had 4 tissue samples for GABA analysis it was not feasible to use 3-way ANOVA to analyse the effects of tumor, DEX, hemisphere and interactions among these three factors; instead 2-way ANOVA was done to compare the effect of tumor and hemisphere within each treatment group. Two-way ANOVA was used to compare the effect of DEX on tumor GABA levels.

a) Tumor

Dexamethasone treatment did not have a significant effect on GABA levels in the different groups of brain tumors.

Table 10. Summary of tumor and dexamethasone (DEX) effects on NA levels in regional brain areas of groups of cats: Control (C) and tumor-bearing (T) groups of cats not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules.

	Parietal Grey		Temporal Grey		Frontal Grey	
	effect	groups	effect	groups	effect	groups
Tumor	decrease	DEX 1, T	decrease	all T	decrease	DEX 1, T
DEX	no change	all groups	no change	all groups	no change	all groups
Tumor-DEX interaction	decrease	DEX 1, T	no change	all groups	decrease	DEX 1, T

Table 11. Summary of tumor and dexamethasone (DEX) effects on DA levels in regional brain areas of groups of cats: Control (C) and tumor-bearing (T) groups of cats not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules.

	Parietal Grey		Temporal Grey		Frontal Grey	
	effect	groups	effect	groups	effect	groups
Tumor	decrease	NO TX, DEX 1; T	no effect	all groups	decrease	all T groups
DEX	no effect	all groups	no effect	all groups	decrease	DEX 2;T,C
Tumor-DEX interaction	increase	DEX 2, T	no effect	all groups	no effect	all groups

Table 12. Summary of tumor and dexamethasone (DEX) effects on DOPAC levels in regional brain areas in groups of cats: Control (C) and tumor-bearing (T) cats not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules.

	Parietal Grey		Temporal Grey		Frontal Grey	
	effect	groups	effect	groups	effect	groups
Tumor	decrease	NO TX, T	no effect	all groups	no effect	all groups
DEX	decrease	DEX 1; C	no effect	all groups	no effect	all groups
Tumor-DEX interaction	increase	DEX 2, T	no effect	all groups	no effect	all groups

Table 13. Summary of tumor and dexamethasone (DEX) effects on HVA levels in regional brain areas in groups of cats: Control (C) and tumor-bearing (T) groups of cats not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules.

	Parietal Grey		Temporal Grey		Frontal Grey	
	effect	groups	effect	groups	effect	groups
Tumor	no effect	all groups	no effect	all groups	no effect	all groups
DEX	increase	DEX 2; T,C	no effect	all groups	decrease	DEX 1;T,C
Tumor-DEX interaction	no effect	all groups	no effect	all groups	decrease	DEX 2; T

Table 14. Summary of tumor and dexamethasone (DEX) effects on 5-HT levels in regional brain areas in groups of cats: Control (C) and tumor-bearing (T) groups of cats not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules.

	Parietal Grey		Temporal Grey		Frontal Grey	
	effect	groups	effect	groups	effect	groups
Tumor	no effect	all groups	no effect	all groups	decrease	all T groups
DEX	no effect	all groups	decrease	DEX 2;T,C	decrease	DEX 2;T,C
Tumor-DEX interaction	no effect	all groups	no effect	all groups	no effect	all groups

Table 15. Summary of tumor and dexamethasone (DEX) effects on 5-HIAA levels in regional brain areas in groups of cats: Control (C) and tumor-bearing (T) groups of cats not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules.

	Parietal Grey		Temporal Grey		Frontal Grey	
	effect	groups	effect	groups	effect	groups
Tumor	no effect	all groups	no effect	all groups	no effect	all groups
DEX	increase	DEX 2;T,C	no effect	all groups	no effect	all groups
Tumor-DEX interaction	no effect	all groups	no effect	all groups	increase	DEX 2,T

b) Parietal Grey Matter

There was no effect of either tumor or hemisphere on GABA levels in either the untreated or DEX 1-treated groups (Fig. 26). There was an overall effect of hemisphere in the DEX 2-treated group [F(1,23)= 5.36, $p < 0.05$]; however, this was not significant in either the tumor-bearing or control group. There was no effect of vehicle-injection on GABA levels.

c) Temporal grey matter

In untreated cats GABA levels were higher in the tumor-bearing cats than in controls [F(1,29)=5.78, $p < 0.05$] (Fig. 26). This was not apparent in cats treated with DEX 1, but tumor-bearing cats treated with DEX 2 also had higher GABA levels than their drug-treated controls [F(1,23)=7.29, $p < 0.05$]. There was no effect of vehicle-injection on GABA levels in this area.

d) Frontal Grey Matter

There was no significant effect of tumor or hemisphere on GABA levels in any of the three treatment groups (Fig. 26). Levels of GABA were also not affected by vehicle injection.

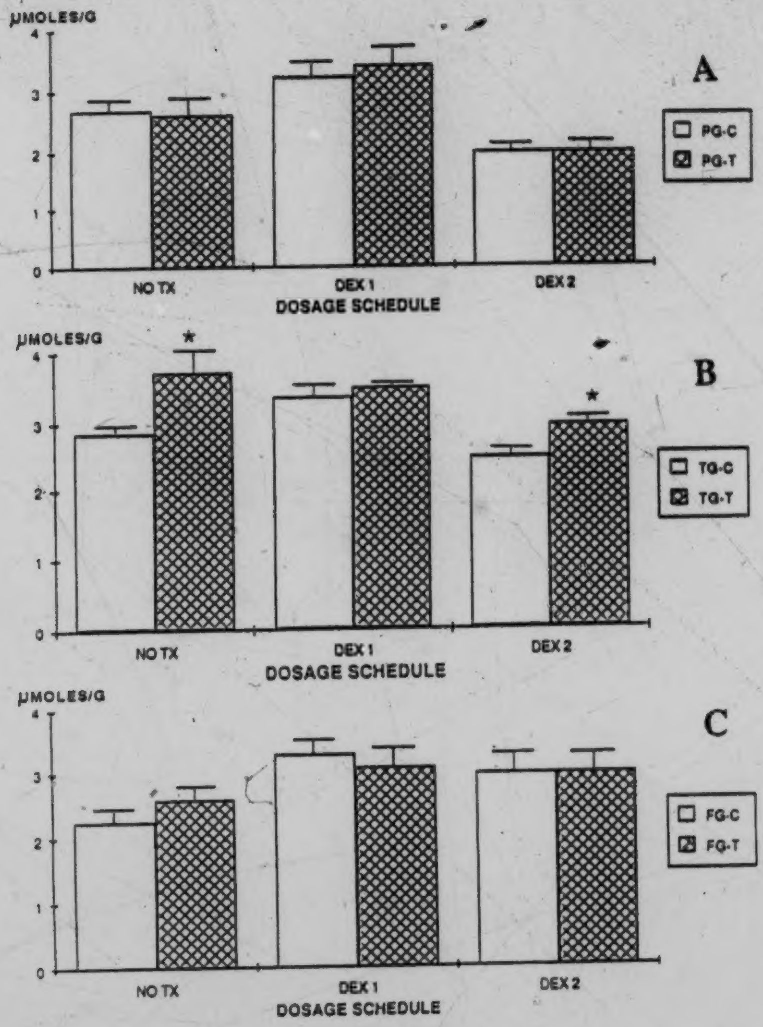


Fig. 26. Levels of GABA (µmoles/g wet weight ± SEM) in the parietal grey (PG) (A), temporal grey (TG) (B) and frontal grey (FG) (C) matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n=4-9/group)

Effect of tumor in each treatment group
* $p < 0.05$

e) Parietal White Matter

In untreated animals, tumor-bearing cats had significantly lower GABA levels than control cats [F(1,26)=12.74, $p < 0.01$] (Fig. 27). This effect was not apparent in either DEX 1- or DEX 2-treated cats. Vehicle-injection had no effect of GABA levels in this area.

f) Frontal White Matter

Levels of GABA were lower in untreated tumor-bearing cats [F(1,24)= 5.19, $p < 0.05$], but in DEX-treated animals this difference was not apparent (Fig. 27). Levels of this amino acid were not significantly affected by vehicle injection.

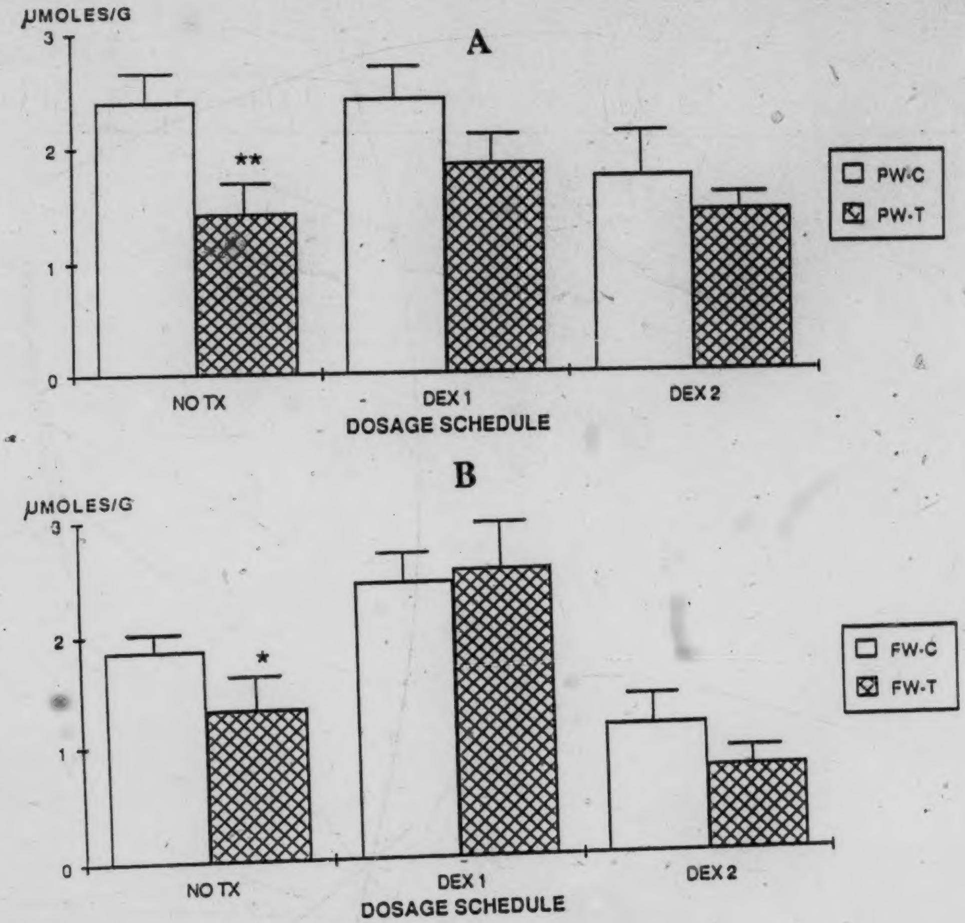


Fig. 27. Levels of GABA (µmoles/g wet weight ± SEM) in the parietal white (PW) (A) and frontal white (FW) (B) matter of tumor-bearing (T) and control (C) animals not treated (NO TX) or treated with DEX 1 or DEX 2 dosage schedules. Levels are pooled from both hemispheres. (n=4-9/group)

Effect of tumor in each treatment group
* $p < 0.05$
** $p < 0.01$

4.3.5 Regional water content levels

a) Tumor

Water content in the tumor was not changed by the administration of either dose of DEX.

b) Grey Matter

Administration of DEX or the presence of the tumor did not alter parietal and frontal grey matter water content. Temporal grey water matter content differed between treatment groups [F(2,48)= 10.66, $p< 0.01$] and there was also a hemisphere-DEX interaction [F(2,48)=6.75, $p< 0.01$]. Water content in control animals did not differ between groups, but tumor-bearing animals in the DEX 1 (83.5 ± 0.2%) and DEX 2 (83.0 ± 0.1%) groups had significantly greater water content than those in the NO TX group (82.0 ± 0.3%, $p< 0.01$). The differences between hemispheres was not significant within groups.

c) White Matter

There was an effect of tumor [F(1,60)=42.10, $p< 0.01$] and hemisphere [F(1,60)=17.16, $p< 0.01$)], with the tumor having a significant effect [F(2,60)=8.98, $p< 0.01$] on water content in peritumor (parietal) white matter. This was not alleviated by

DEX treatment. Per cent water content was 73.8 ± 2.2 (mean ± SEM) in untreated parietal white matter, 68.6 ± 0.9 in the contralateral hemisphere and 68.0 ± 1.0 in the equivalent area in control brain. Values were similar in the DEX-treated animals.

Raised water content was also recorded in frontal white matter of the tumor-bearing hemisphere. There was an overall effect of tumor [F(1,36)= 14.64, p< 0.01] and hemisphere [F(1,36)= 9.68, p< 0.01], with a significant tumor-hemisphere interaction [F(1,36)= 6.59, p< 0.01], although it was not significant in untreated tumor-bearing animals. Per cent water content in this area was similar to the values in parietal white matter. DEX treatment had no effect on water content in the tumor, grey or white matter.

4.3.6 Conclusions

As the results of this study showed, the presence of a brain tumor altered levels of neurotransmitter amines and their metabolites in the surrounding brain of cats without abnormal neurologic signs of increased ICP, thus confirming the findings of study B. Although NA and 5-HT concentrations were also lowered, levels of DA and its metabolite DOPAC were most severely decreased, especially in brain regions adjacent to the tumor.

Administration of DEX had profound effects on neurotransmitter amines and their metabolites in both control and tumor-bearing brain. In peritumor grey matter, treatment with DEX for 20 h (DEX 2) led to a normalization of depressed DA and DOPAC levels despite the fact that DEX treatment did not alleviate white-matter edema.

4.4 Study D

Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites and GABA, and Water Content in Tumor-bearing Brain of Cats with Abnormal Neurologic Signs: the Effect of DEX 1 Dosage Schedule

4.4.1 Neurologic status

Animals with anisocoria and somnolence were assigned to a no treatment (n=8) or DEX 1 treatment schedule (n=5). Four of the five treated animals showed an improvement in neurologic status after 6 h of DEX 1 treatment (Table 16). Untreated animals did not improve. Biochemical measurements could be done on 6 untreated animals and 4 treated animals. One animal died before in vivo freezing and two animals were euthanized and the brains evaluated histologically. Arterial blood pressure, pH, pCO_2, and pO_2 measured immediatedly prior to sacrifice were not different between the two groups.

4.4.2 Intracranial pressure

The ICP was measured immediately prior to in vivo freezing of the brain. In the untreated cats the ICP was 13.5 ± 0.5 mm Hg while the treated cats had a mean ICP of 10.0 ± 1.5 mm Hg (no significant difference).

Table 16. Cats with neurologic signs of increased ICP-No Treatment (N-NO TX)

Neurologic Status Immediately Prior to Anesthesia	Score	#Cats
	3	1
	2	6
	1	1*

*died before further neurologic assessment could be done

Changes in neurologic scores of cats with neurologic signs of increased ICP after initial treatment with dexamethasone (0.25 mg/kg i.v., 0.25 mg/kg i.m.) (N-DEX 1)

	Time Post-injection (h)			
Cat#	0	2	4	6**
1	3	3	3	3
2	2	0	0	0
3	2	2	1-2	0
4	2	1	0	0
5	2	2	2	1

**immediately prior to anesthesia

4.4.3 Regional levels of neurotransmitter amines and metabolites

a) Tumor

Neurotransmitter amine and metabolite levels were not different between these two groups of tumor-bearing animals.

b) Parietal Grey Matter

Levels of NA, DA, 5-HT, DOPAC, and 5-HIAA were not different in the treated cats compared to the untreated animals. Levels of HVA were higher in treated animals than in untreated animals [F(1,19)=9.09, $p< 0.01$] (Table 17).

c) Temporal Grey Matter

Levels of NA were increased in treated animals compared to untreated animals [F(1,19)= 7.37, $p< 0.05$]. Dopamine and the metabolites DOPAC and HVA were unchanged although HVA levels showed a tendency to increase in the treated group [F(1,19)=3.43, $p< 0.10$] (Table 18).

Table 17. Neurotransmitter amine and metabolite levels (ng/g wet weight) in the parietal grey matter of the tumor-bearing (R) and contralateral (L) hemisphere of cats with abnormal neurologic signs not treated (N-NO TX) or treated with dexamethasone (N-DEX 1) (mean ± SEM, n=4-6)

	NA	DA	5-HT	DOPAC	HVA	5-HIAA
N-NO TX						
R	165±17	45±5	280±33	2±1	60±12	155±26
L	188±27	68±14	269±44	4±3	64±23	164±35
N-DEX 1						
R	200±27	66±17	304±26	4±4	180±58*	179±59
L	193±26	66±8	259±53	10±9	117±31*	113±21

* $p < 0.05$

Table 18. Neurotransmitter amine and metabolite levels (ng/g wet weight) in the temporal grey matter of the tumor-bearing (R) and contralateral (L) hemisphere of cats with abnormal neurologic signs not treated (N-NO TX) or treated with dexamethasone (N-DEX 1) (mean ±SEM, n=4-6)

	NA	DA	5-HT	DOPAC	HVA	5-HIAA
N-NO TX						
R	248±31	62±11	560±130	4±2	45±11	327±62
L	231±23	76±13	234±26	21±11	41±7	150±24
N-DEX 1						
R	343±32*	134±64	429±157	14±9	105±66†	303±123
L	333±47*	108±38	428±27	9±6	92±27†	250±17

† $p < 0.10$

* $p < 0.05$

d) Frontal Grey Matter

Levels of NA and DA and its metabolites DOPAC and HVA were not altered due to DEX treatment (Table 19). There was a overall hemispheric effect [$F(1,19)=4.74$, $p < 0.05$] on 5-HT levels, but sides were not significantly different in either the untreated or the treated groups. The levels of the 5-HT metabolite 5-HIAA were not altered by the drug treatment (Table 19).

4.4.4 Monoamine oxidase inhibition

A short course of DEX treatment (DEX 1) did not alter *ex vivo* inhibition of MAO-A or MAO-B in the brains of cats with abnormal neurologic signs.

4.4.5 Regional GABA levels

a) Tumor

Dexamethasone treatment did not alter GABA levels although there was a trend towards decreased GABA levels ($p < 0.10$) in treated cats.

Table 19. Neurotransmitter amine and metabolite levels (ng/g wet weight) in the frontal grey matter of the tumor-bearing (R) and contralateral (L) hemisphere of cats with abnormal neurologic signs not treated (N-NO TX) or treated with dexamethasone (N-DEX 1). (mean±SEM, n=4-6)

	NA	DA	5-HT	DOPAC	HVA	5-HIAA
N-NO TX						
R	298±20	110±13	228±35	51±3	128±26	197±13
L	387±52	148±39	368±72	60±15	134±50	148±39
N-DEX 1						
R	302±28	142±34	236±32	56±13	196±26	205±31
L	356±25	126±10	298±32	31±9	105±6	194±11

b) Grey Matter

Levels of GABA in parietal and temporal grey matter were not different in untreated and DEX-treated animals. In frontal grey matter GABA levels were lower in DEX-treated cats [$F(1,19)=4.39$, $p < 0.05$] (Table 20).

c) White Matter

Levels of GABA were not different in either the parietal white matter or in frontal white matter of DEX 1 treated cats compared to untreated cats.

4.4.6 Regional water content levels

a) Tumor

Water content in the tumor was the same in the two groups.

b) Grey matter

Grey matter water content in parietal, temporal, and frontal areas was not altered by DEX treatment. Water content levels were comparable to levels in control brain and in brain of tumor-bearing animals without neurologic signs.

Table 20. Levels of GABA (µmoles/g wet weight) in parietal grey (PG), temporal grey (TG), frontal grey (FG), parietal white (PW) and frontal white (FW) matter in the tumor-bearing (R) and contralateral hemisphere of cats with abnormal neurologic signs not treated (N-NO TX) or treated with DEX 1 dosage schedule (N-DEX1) (mean±SEM, n=4-6).

	PG	TG	FG	PW	FW
N-NO TX					
R	4.51±.54	3.23±.26	6.04±.72	2.25±.42	1.71±.40
L	4.02±.49	3.09±.35	4.43±.65	1.82±.14	2.31±.34
N-DEX 1					
R	3.78±.14	3.72±1.00	3.67±.60*	2.49±.43	1.59±.45
L	4.31±.52	3.24±.63	3.74±.32*	2.83±.53	2.97±.54

$^*p < 0.05$

c) White matter

Water content in parietal [F(1,19)= 43.14, $p < 0.01$] and frontal white matter [F(1,19)= 16.66, $p < 0.01$] was elevated in the tumor-bearing hemisphere compared to the contralateral hemisphere. The water content in parietal white matter in the tumor-bearing hemisphere of untreated animals was 75.0 ± 1.6 while the water content of the contralateral hemisphere was 67.8 ± 0.5. Water content in frontal white matter was very similar. There were no differences in water content between untreated and treated animals.

4.4.7 Conclusions

In animals with neurologic signs of increased ICP, DEX 1-treatment caused an improvement in neurologic status over a 6 h period. Treatment appeared to reverse some of the neurochemical abnormalities that were seen in tumor-bearing cats, both with and without abnormal neurologic signs. Levels of HVA were increased in the parietal grey matter of treated animals and NA levels were elevated in temporal grey matter. Decreased levels of GABA were recorded in frontal grey matter of treated animals. DEX 1-treatment in these cats did not lower the increased water content measured in parietal and frontal white matter.

CHAPTER FIVE DISCUSSION

5.1 Study A

Characteristics of a Feline Brain Tumor Model

One conclusion from the research just described is that the feline glioma tumor model is well suited for studies evaluating regional biochemical changes associated with the tumor and peritumor edema. Histologically, the tumor shared many of the characteristics of human gliomas and glioblastomas, including numerous mitotic figures and occasional multinucleated giant cells together with vascular proliferation; also, the glial proteins PTAH and GFAP were present, but S-100 protein was not. Although this tumor cell line has been classified as a gliosarcoma (Rubinstein, 1977), trichrome staining demonstrated no collagen, which is diagnostic of a gliosarcoma. In addition, the gross and histologic changes in peritumor brain tissue were very similar to those in naturally occurring tumors. There was extensive white matter edema in the tumor-bearing hemisphere, displacing the midline in many cases. The vasogenic nature of the edema was established by earlier findings in our laboratory (Castro, 1986) of marked increases in protein and albumin within the edematous white matter. The negative correlation between increased tumor size and decreased white matter water content in cats with abnormal neurologic signs, which was unexpected, could be due to compression of adjacent white matter. This effect was also seen by Castro (1986), who found that edema was less in tissue adjacent to the tumor than in tissues slightly further away. The tumor 'take' was predictable and reproducible, with a high success rate. Time of onset of clinical signs was fairly consistent, allowing treatment protocols to be planned for a specific day and time after implantation.

One disadvantage of this model is the variable size of the tumor and associated edema on a specific day after implantation. A major factor governing the irregular tumor growth in transplanted brain tumors is the implantation technique. The tumor cell suspension must be placed sterotactically in the brain because duration of survival of the animals depends substantially on both the location and localization of the tumor; if the tumor cell suspension refluxes up the needle track, it can lead to meningeal tumor growth (Rama et al., 1986). Injection into grey matter can lead to the production of small tumors; however, it produces little edema, confirming the finding by Clasen et al. (1962) that grey matter is markedly resistant to the spread of edema.

The use of this feline model for the study of peritumor metabolism and edema in humans has been criticized, for three reasons: the BBB characteristics of the tumor are different from human gliomas; the implantation procedure disrupts the BBB; and the implantation of xenogenic tissues into an immunocompetent host leads to an immunologic response. This may disrupt the BBB and could confound the anti-edema effects of immunosuppressive drugs (i.e. DEX).

The suitability of the 9L and other chemically derived glioma cell lines for chemotherapeutic studies has been questioned because of the variable permeability of the BBB in the brain tumor (Levin et al., 1975; Vick et al., 1977; Groothuis et al., 1982). On the other hand, other authors feel that this variability is an important factor in the chemotherapy of human gliomas (Benjamen et al., 1974; Neuwelt et al., 1984). Vick et al. (1977) felt that the injection procedure may disrupt the BBB although this has not been seen by other investigators (Ausman et al., 1970; Groothuis et al., 1982).

The lymphocytic infiltration around the tumor could be interpreted as a rejection response that might compromise the peritumor BBB. However, the marked rejection response usually seen with brain xenografts or allografts was absent; instead, the scattered perivascular cuffing of lymphocytes more closely resembled that seen during histologic assessment of human glial tumors (Ridley et al., 1969, 1971; Takeuchi et al., 1976; Brooks et al., 1978; Scheinberg et al., 1984). Lymphocytic infiltration was also seen in the peritumoral white matter of non-immunosuppressed cats implanted with RG2 gliomas (Ebhardt et al., 1982) and horseradish peroxidase studies revealed an intact BBB in that area.

Saris et al. (1984) considered that immunosuppressed animals were appropriate for brain-tumor studies because patients with tumors of glial origin had depressed cell-mediated immunity

(Kumar et al. 1973; Menzies et al., 1980). It has also been demonstrated, however, that the immunologic response in patients with brain tumors is linked to response to therapy (Brooks et al., 1978). This suggests that immunocompetent animals may be more appropriate for brain-tumor studies.

Rejection of tumor cells may have been one of the causes of implantation failure, although histologic evaluation revealed only chronic brain injury consistent with the time of implantation. Other possible causes include technical errors in the preparation, handling, or injection of the cells. Implantation of the 9L glioma into the isologous host, the Fisher 344 rat, would eliminate the problem of xenotransplantation. Use of a smaller host such as a rat killed by decapitation would have eliminated the need for anesthesia and would be more economical. On the other hand, the brain size of the cat allowed the tumor to grow to a considerable size in the white matter without causing the massive cortical and midbrain destruction generally seen in rats implanted with this tumor. The larger size made it possible to take several samples (> 20 mg) from each brain region thus increasing the ability to correlate changes in water content with neurotransmitter alterations. It was felt that the advantages of several biochemical analyses from multiple areas of the cortex offset the disadvantages of increased cost per animal, and more complex design and methodology.

A larger animal model using an autochthonous brain tumor such as avian sarcoma virus or JC virus would eliminate the criticisms associated with transplantable tumor models. These models, however, generally have longer and more variable latencies (months) and involve the use of primates or immature animals such as beagle puppies (see chapter 1.2.1). Immature animals were unsuitable for this study because of their variable brain myelination, neurotransmitter levels, and water content (Agrawal et al., 1967; Keller et al., 1973; Brockbank et al., 1978; Kinney et al., 1988).

In summary, the transplantable brain-tumor model was appropriate for this experimental study since 1) it closely approximated the naturally occurring condition and 2) it was predictable and reproducible with a short latency, therefore providing the numbers of experimental animals adequate to address the problems posed in this study.

5.2 Study B

Neurologic Status, Levels of Neurotransmitter Amines, their Metabolites, and GABA in Untreated Tumor-Bearing Brain

The results of this study revealed bilateral differences in the levels of cortical monoamines, their metabolites, and GABA in the cortex of tumor-bearing brains. There were marked decreases in DA and its metabolites in cortex nearest the tumor; NA was decreased in more remote cortical areas. These changes were more severe in cats with neurologic signs of increased ICP. Because grey matter contained little edema, the observed changes in neurotransmitter and metabolite levels were not simply because of dilution by edema fluid, but probably reflected altered metabolism of the neurotransmitters.

The techniques of tissue collection and methods of determining brain levels of monoamines, metabolites, and GABA used in this study made it possible to obtain accurate results and draw certain conclusions from the information. Freezing in vivo is currently the most acceptable method of collecting brain tissue samples for biochemical studies from larger animals (Welsh & Rieder, 1978; Yang et al., 1983) because it preserves energy metabolites such as ATP and AMP, which are sensitive indicators of tissue hypoxia. Microwave irradiation would have been a better choice for monoamine and GABA studies because it prevents postmortem degradation (Ikarashi et al., 1985); however, a microwave oven large enough for cats is not available. Monoamines are thought to remain stable in whole brain for at least 8 months if stored at -70°C (Spokes, 1979), although levels of monoamines in small samples decrease over time, possibly due to prolonged exposure to oxygen before processing (Wiesal et al., 1974; Slouter et al., 1977; Faull et al., 1988).

The methods used for tissue dissection and sampling are similar to the techniques used for dissecting human brain postmortem for neurochemical studies (Bird & Iversen, 1982; Langlais et al., 1983). Ideally, the brains would be cut while still frozen but, even if the blade is irrigated with liquid nitrogen, the mechanical saw thaws the tissue and thus obscures the demarcation between grey and white matter. In addition, Langlais et al. (1983) reported cutting with a rotating serrated blade seriously decreased regional NA and DA levels in frozen human brain tissue.

For this study, analytical methods appropriate for measuring of low levels of compounds in large numbers of tissue samples of less than 30 mg were required. The monoamines NA, DA, and 5-HT and the acid metabolites DOPAC, HVA and 5-HIAA are most easily and accurately measured using HPLC with electrochemical detection (Kim et al., 1983; Kontur et al., 1984; Ehrenstöm & Johansson, 1985). Detector sensitivity is high and all six compounds can be measured concommitantly.

The GC assay used to measure GABA levels is simple, rapid, sensitive, and relatively inexpensive (Yeung et al., 1986). It is also suitable for automation since the derivatized samples remain stable for at least 24 h at room temperature. A disadvantage of this method is its inability to separate glutamate from glutamine and aspartate from asparagine. Glutamate and aspartate are excitatory amino acids also considered important as neurotransmitters in the CNS (reviewed by Fagg, 1985). Precolumn derivatization of amino acids followed by separation by reversed-phase HPLC separates GABA, glutamate, and aspartate quickly and with high sensitivity However, instability of the derivitives makes automation difficult without expensive ancillary equipment and an HPLC system dedicated to amino acid analysis (Gardner & Miller, 1980; Chapman et al., 1982; Ellison et al., 1987).

Mononamine and metabolite levels in 9L gliomas were consistent with levels (ng/g) recorded in experimental (Ikeda et al., 1984) and human gliomas (Ikeda & Nakazawa,1984). The cultured 9L tumor cells also had appreciable levels of monoamines and metabolites except that, suprisingly, there was no observable 5-HT. Detectable levels of monoamines and metabolites would be expected, because the cells were grown in fetal calf serum and would take up monoamines and metabolites from this source (Henn et al., 1971). GABA levels in the tumors and tumor cells were also in accordance with previously published data (Mokrasch, 1971; Lefauconnier et al., 1976; Shibasaki et al., 1979).

Levels of cortical monoamines and metabolites were comparable to those measured by Reader et al. (1979) in the cat and similar to regional cortical levels in nonhuman primates (Goldman-Rakic & Brown., 1981; Haggstrom et al., 1984; Dewhurst, personal communication) and man (Langlais et al., 1983). Although cortical dopaminergic tracts are considered to be confined to the prefrontal cortex in the rat, histochemical and neurochemical data indicate a more extensive dopaminergic mesocortical system in carnivores and primates (reviewed by Oades & Halliday, 1987). Levels of GABA were also similar to those found in another study in cats (Tallen et al., 1954), although higher than biopsied feline cortical tissue (Perry et al., 1972) and were equivalent or slightly higher than regional levels in nonhuman primates (Fahn & Côté, 1968; Dewhurst, personal communication) and man (Perry et al., 1971a, 1971b; Ellison et al., 1987).

Neurochemical alterations in peritumor brain tissue have been reported by others. Chang et al. (1988) found decreased cortical NA and striatal DA levels in rats implanted with 9L glioma tumors; however, changes in cortical NA may have been related to cortical edema. Von Metzlar & Nitsch (1985) found that transplanted (Walker carcinomas) and chemically induced (3-MC) brain tumors decreased the regional brain concentrations of NA, DOPAC, and 5-HIAA (they did not measure DA, 5-HT, and per cent water content); in an earlier study (1981) they found that 3-MC induction increased the GABA levels in the hypothalamus and hippocampus during the carcinogenic period. These findings of neurochemical alterations are in accordance with other reports of depressed cerebral metabolism in peritumor brain. Using PET, studies in patients with brain tumors have demonstrated decreased rCBF and decreases in both oxygen and glucose metabolism in peritumor tissue (Di Chiro et al., 1982; Tsubokawa et al., 1985) as well as in more remote regions (Ito et al., 1982; DeLaPaz et al., 1983; Taki et al., 1985). The rOER in peritumor tissue was normal, indicating a reduced metabolic rate (Ito et al., 1982; Taki et al., 1985; Tsubokawa et al., 1985). Regional CGU has been reported to be unilaterally or bilaterally depressed in brain tumor models (Altenau et al., 1977; Shinohara et al., 1979; Hossmann et al., 1982, 1986), and in other forms of brain injury (Pappius, 1981, 1982). Reduced peritumor tissue metabolism, reflected by decreased concentrations of energy-rich phosphates (Reulen *et al.*, 1967) and increased lactate in human grey matter, has been reported (Reulen *et al.*, 1967; Schmiedek *et al.*, 1974).

Similar alterations in monoamines and their metabolites, and in GABA, occur in other brain insults. Generally, insults causing cytotoxic or ischemic edema rapidly deplete monoamines but increase levels of metabolites (Mrsulja *et al.*, 1976; Lloyd *et al.*, 1977; Matsumoto *et al.*, 1984; Bentue-Ferrer *et al.*, 1986; Baran *et al.*, 1987) Decreased monoamine concentrations are probably due to release from presynaptic terminals (Phebus *et al.*, 1986; Brannan *et al.*, 1987), whereas metabolites are thought to accumulate because of increased synthesis or turnover, decreased degradation, and/or washout (Mrsulja *et al.*, 1976). In this study decreased levels of monoamines were observed but no increases in metabolites except 5-HIAA. Increased levels of 5-HIAA in the parietal cortex of tumor-bearing cats with abnormal neurologic signs were probably due to minor hemorrhages in and around the tumor. Increased levels of 5-HIAA were also found in the corresponding tumor tissue. Alternatively, increased levels of 5-HIAA may indicate increased 5-HT turnover associated with tissue damage. Similar increases in 5-HIAA, and decreases in 5-HT, have been seen in the lesioned hemisphere following focal freezing brain injury with associated vasogenic edema (Pappius & Dadoun, 1987), but no alterations in DA or its metabolites were observed (Pappius & Dadoun, 1986).

Alterations in GABA levels have been studied in brain of patients with cerebral infarcts (Jellinger et al., 1978). Levels were increased in areas adjacent to the infarct, presumably due to ischemia.

Depletion of cortical monoamine neurotransmitters could occur through various mechanisms including loss or dysfunction of cortical synaptic input and/or increased release or decreased synthesis of neurotransmitters at the nerve terminal. Peritumor white matter edema may impair neuronal transmission in the NA, DA, and 5-HT tracts which originate in the midbrain and brainstem and terminate in the cortex. Marked increases in water content were present in white matter adjacent to peritumor grey matter that contained depleted levels of DA and its metabolites. In contrast, no changes in DA, DOPAC, and HVA were seen in frontal grey matter where there was no significant adjacent white matter edema. These findings suggest a role for the edema in producing the neurotransmitter and metabolite depletions. Alterations in NA, 5-HT, and 5-HIAA were less consistent than in DA and its metabolites.

The majority of cortical GABA neurons are short interneurons with both cell body and presynatic terminal located in the same cortical region. Any alterations in GABA levels would probably be due to changes in the local environment, although interactions with dopaminergic neurons are also important (Bonnano & Raiteri, 1987). Grey matter GABA levels were increased most dramatically in cats with neurologic signs of increased ICP, suggesting that local compression of tissues and perhaps ischemia may have been a factor. Decreased GABA levels in parietal white matter of tumor-bearing animals without abnormal neurologic signs was proportional to the degree of edema in the tumor-bearing hemisphere. This implies that the observed decreases were simply due to dilution, although similar changes were also seen in the non-edematous hemisphere. There was an equivalent amount of edema in cats with abnormal neurologic signs, but leakage of extracellular GABA from adjacent parietal grey matter (which had increased GABA levels) may have raised the levels in white matter. This could also explain the increased GABA levels found in the tumors of this group of cats.

Bilateral decreases in neurotransmitter and metabolite levels would be consistent with a depressing effect of the tumor and surrounding edema on neuronal impulse transmission and cortical function. Although the issue remains controversial, there is strong evidence that electrocortical function in adjacent and remote peritumor brain tissue is impaired (see chapter 1.3.5). In experimental peritumor brain edema, Fourier transform of the EEG shows significant slowing of the mean frequency on the affected side. Hossmann et al. (1979) found no correlation between EEG changes and tumor size or time since implantation, water or electrolyte content of grey or white matter, blood flow or ICP. These investigators and others have postulated that the EEG changes are due to direct effects of the tumor on brain parenchyma, rather than peritumorous edema or intracranial hypertension (Gaustat et al., 1979; Hossmann et al., 1979; Penn et al., 1980).

Neurotransmitter alterations in the contralateral hemisphere may be due to a diaschisis effect. Although the precise mechanisms are unknown, loss or impairment of transynaptic input is widely believed to account for depressed metabolism in regions remote from a brain lesion (reviewed by Feeney & Baron, 1986). This effect has been seen in tumor-bearing brain (Ito et al., 1982; DeLaPaz et al., 1983) as well as in other brain insults causing edema (Reivich et al., 1977; Pappius et al., 1983).

In some animals, an additional factor, namely ischemia secondary to increased ICP, must be considered. Under these conditions, decreased synthesis of monoamines and accumulation of GABA could occur. The synthetic enzymes tyrosine hydroxylase and tryptophan hydroxylase have an absolute requirement for molecular oxygen (Daly et al., 1968) and any decrease in the oxygen supply may slow the rate of transmitter synthesis. Relatively mild hypoxia (10% O_2) in rats significantly reduces synthesis of catecholamines and 5-HT (Davis and Carlsson, 1973; Davis et al., 1973), and tryptophan hydroxylase activity decreases at PaO_2 values that do not affect tissue lactate concentrations and adenylate energy charge (Davis et al., 1973). However, the major alterations observed in this study were again restricted to DA and its metabolites.

Levels of GABA are also altered by changes in tissue O_2 concentrations. Tissue GABA levels rise in ischemia and hypoxia (Wood et al., 1968; Folbergrova et al., 1974; Norberg & Siesjö, 1975b; Erecinska et al., 1984). Accumulation may occur because of decreased activity of the enzyme GABA-T, which is responsible for degradation of GABA. The function of GABA-T is dependent upon the availability of α-ketoglutarate and NAD^+, both of which are decreased in hypoxia and ischemia (Folbergrova et al., 1974; Norberg & Siesjö, 1975a; Nordstrom & Siesjö, 1978). This may explain the increased GABA levels in cats with increased ICP.

If monoamine depletion in this model is due to decreased activity of synthetic enzymes and/or impaired afferent transmission of impulses to the cortex, why are all three monoamines not equally affected in each brain region? The limitation of alterations to areas with adjacent white matter edema suggests that distortion of white matter tracts may impair neuronal transmission; similar alterations have been seen in brains with hydrocephalus (Chovanes et al., 1988). In a more extensive study (study C), tumor-induced decreases in NA, DA, and 5-HT occurred in all three cortical regions, indicating that all three monoamines are affected to varying degrees. The nature of the monoamine depression may relate to the type of insult: noradrenergic and dopaminergic neurons appear more susceptible to ischemia than do serotonergic neurons, whereas there are major selective changes in DA turnover in response to stressful situations (Fadda, 1978; Reinhard et al., 1982; Weinberger et al., 1983; Kramarcy et al., 1984; Masumoto et al., 1984). Alternatively, it has been shown that there are regional cortical differences in monoamine alterations after stress; this could occur in other pathologic conditions (Herman et al., 1982)

In summary, the observed neurotransmitter alterations in peritumor brain are consistent with others' findings and with reports of decreased cerebral metabolism in experimental models and in patients with brain tumors. The mechanisms for these changes are not understood, but some tentative causes have been discussed.

5.3 Effect of Dexamethasone on Tumor-Bearing Brain

5.3.1 Study C

Neurologic status, levels of neurotransmitter amines, their metabolites and GABA, and watercontent in tumor-bearing brain of cats without abnormal neurologic signs: the effect of two dosage schedules of dexamethasone

Treatment with DEX reversed some of the neurotransmitter alterations seen in tumor-bearing animals, with levels of DA and its metabolites being most affected. In cortex adjacent to the tumor (parietal grey matter), DA and its metabolite DOPAC were decreased due to the tumor; after DEX 2 treatment DA levels were not different from drug-treated controls and DOPAC levels had increased. Levels of HVA also increased, although this was not significantly different from drug-treated controls (see Tables 11, 12, 13).

Opposite effects were seen in more remote grey matter: in temporal grey matter, DA, DOPAC, and HVA levels were not altered by either the tumor or DEX whereas in frontal grey matter DEX 2 treatment caused a decrease in DA and HVA, especially in tumor-bearing brain (see Tables 11, 12, 13). The reasons for these differences are unknown.

Levels of NA were generally decreased by the tumor in all three brain regions, with the effect seen most clearly in DEX 1-treated cats (see Table 10). The tumor size in this group of animals was slightly larger (not significant) than tumors in the untreated or DEX 2 groups, implying that tumor size may affect the degree of neurotransmitter alterations. Dexamethasone did not have a salutary effect on NA changes in tumor-bearing brain. There is no clear explanation for the decreased NA levels in the tumors of DEX 2-treated cats.

In brain regions remote from the tumor, 5-HT levels were decreased (see Table 14). Administration of DEX did not change this-- instead, DEX 2 treatment lead to further decreases of 5-HT in both control and tumor-bearing cats, with increases in its metabolite 5-HIAA (see Tables 14, 15). Because the elevation in 5-HIAA was most apparent in brain tumor and surrounding brain, the presence of the tumor may have also increased levels, resulting in an additive effect.

Levels of GABA were only increased in temporal grey matter of both untreated and DEX 2-treated tumor-bearing cats without abnormal neurologic signs. Transtentorial compression due to white matter edema would tend to be maximal in this brain region, possibly causing areas of focal ischemia with resultant elevations in GABA levels. Levels of GABA were not significantly altered in the brain of DEX 1-treated tumor-bearing cats or in tumor-bearing cats with abnormal neurologic signs. This is unexpected, but small differences in the technique of in vivo freezing could lead to variable levels of GABA within groups. It is known that GABA is exquisitely sensitive to even a short time period of hypoxia (Norberg & Siesjö, 1976b) and the temporal regions would be frozen later than parietal or grey matter.

5.3.2 Study D

Neurologic status, levels of neurotransmitter amines, their metabolites and GABA, and water content in tumor-bearing brain of cats with abnormal neurologic signs: the effect of DEX 1 dosage schedule

Neurologic signs were alleviated in animals treated with a DEX 1 dosage schedule, agreeing with clinical observations of early neurologic improvement in patients with brain tumors treated with DEX (see sections 1.6.1, 1.6.2, 1.6.3). In parietal grey matter, HVA levels, which were decreased from controls in untreated cats (study B), were higher in cats treated with DEX, although DA levels remained depressed. In temporal grey matter, NA levels, which were decreased in untreated animals (study B), were also increased. Concentrations of GABA in frontal grey matter were decreased in treated animals.

Improvements in neurologic status and some neurochemical indices were not related to any decrease in brain edema or intracranial pressure at sacrifice. It would have been useful to determine changes in ICP over the 6 h period preceding anesthesia as this would be more reliable as an indicator of changes in the frequency of plateau waves or the value of resting ICP; however, continous monitoring would have necessitated either the use of anesthesia, interfering with neurologic assessment, or the use of chronically instrumented animals, complicating the interpretation of the neurochemical results.

5.3.3 Summary

The main purpose of these two studies was to determine whether administration of DEX normalized neurotransmitter alterations in peritumor brain; a minor objective was to evaluate the effects of DEX in normal brain. The observed changes in peritumor monoamines due to DEX were consistent with a previous study. Chang et al. (1988) observed bilateral decreases of NA in the cortex and DA in the striatum of tumor-bearing rats and restoration of these levels to control values after 2 days of DEX treatment (20 mg/kg/day). Their study differs from these studies (C and D) in the length of treatment, drug dose, the use of rats as experimental animals, and in that the animals had signs of increased ICP at the time of sacrifice or treatment. Chang et al. (1988) reported that treatment with DEX resolved peritumor cortical edema (rat cortex generally contains some white matter); these changes may have led to the increased NA levels seen in the drug-treated animals. Sham-operated rats did not have any monoamine alterations due to the surgery or drug.

Other neuroactive drugs may produce a similar effect. Von Metzlar & Nitsch (1985) found that transplanted (Walker carcinoma) and chemically induced (3-MC) brain tumors caused decreases in NA, DOPAC, and 5-HIAA in various brain regions; in this investigation DA and 5-HT were not measured. Treatment with the neuroactive drugs piracetam and imipramine during the period of 3-MC carcinogenesis prevented these decreases as well as inhibiting tumor growth.

The glucocorticoid DEX may alter monoamine and metabolite levels through various mechanisms. Glucocorticoids either directly or indirectly induce activity of the synthetic enzyme tyrosine hydroxylase and also increase levels of the second messenger cAMP in neurons and glia (Hanbauer et al., 1975; Thoenen et al., 1978; Haycock et al., 1982; Tank & Ham, 1984). The reuptake of DA into synaptosomes is inhibited by DEX and DEX may also inhibit MAO activity; this latter effect would tend to increase levels (Hellstrom et al., 1979; Gilad et al., 1987). Decreased levels of DOPAC and HVA have been seen after 4 h of treatment with DEX (Rothschild et al., 1985); a similar effect was also seen in control animals after DEX 1-treatment in this study. Increased levels of metabolites seen in parietal grey matter after DEX 2-treatment may be a reflection of increased synthesis of DA with increased turnover. In addition, stress (cortisol release) increases DA synthesis and turnover in the cortex; interaction of DEX with cortisol receptors may produce the same effect (Reinhard et al., 1982).

Decreased 5-HIAA levels due to DEX treatment would be expected since both 5-HT turnover and 5-HIAA levels are generally decreased by DEX (Kawamura et al., 1984; Rothschild et al., 1985; Bayens-Simmonds et al., 1986); however, an increased 5-HIAA/5-HT ratio in DEX 2-treated animals may indicate a biphasic response to the drug (Hall & McGinley, 1982).

In addition to its direct effects on monoamine synthesis, DEX may act indirectly to normalize neurotransmitter levels by improving rCBF or energy metabolism (Reulen et al., 1972; Mies et al., 1983; Yamada et al., 1983). A recent study by Leenders et al. (1985) found, however, that DEX decreased rCBF in all brain regions except edematous areas. Glucocorticoids can improve rCGU and energy charge in injured or ischemic brain within the first 24 h of therapy (Pappius et al., 1982; Kuwachi et al., 1985) although DEX does not alter rCGU in normal brain. These indirect effects could explain why neurotransmitters are sometimes changed only in tumor-bearing brain. Levels of monoamines and GABA are O_2- and energy- dependent, so increases in blood flow and energy utilization would tend to improve neurotransmitter metabolism in areas most affected by the tumor.

Ideally, untreated cats with neurologic signs would have been treated with vehicle and observed for exactly the same time period as DEX 1-treated cats (study D). Since animals did not develop neurologic signs at a predetermined time it was not always possible to sacrifice untreated cats exactly 8 h after the first onset of clinical signs although all cats were sacrificed between 11:00 and 16:30 on the day of the study. Previous observations in our laboratory indicated that untreated cats did not improve neurologically once they had anisocoria; instead, they deteriorated and died within 12 h. Because it had been demonstrated that injection of vehicle did not alter neurochemical levels in control animals, it was felt that this omission did not seriously affect the validity of this study. Control tissues analyzed at the same time as these tumor-bearing animals had values comparable to control values recorded in study B. This allowed direct evaluation of the effect of clinical signs on neurochemical alterations in the tumor-bearing brain.

Why were there some indications of a change in neurochemical parameters towards control levels in animals with abnormal neurologic signs treated with DEX 1 (8 h course of therapy) whereas animals without abnormal neurologic signs showed positive changes only after 20 h of therapy? Although there were some improvements in neurochemical levels, these were not extensive and the most severe change, depressed DA levels in peritumor brain, was not alleviated. Neurotransmitter alterations were more severe in these animals; treatment may only have raised levels to values in animals without abnormal neurologic signs treated with DEX 1. Additionally, DEX-altered

VPR or amplitude of plateau waves may have an effect on neurotransmitter levels in cats with increased ICP.

In summary, treatment with DEX for 20 h appeared to normalize monoamine alterations in regions adjacent to the tumor. Earlier improvements in levels may be apparent in animals with abnormal neurologic signs of increased ICP. These improvements may be due to the direct effect of DEX on neurotransmitter metabolism; alternatively, DEX-induced increases in CBF, energy metabolism and glucose metabolism may provide a more normal environment for neurotransmitter synthesis.

Finally, the improvements in peritumor neurotransmitter levels after DEX treatment were not the result of decreased white matter edema. This is consistent with the widely held belief that the early (< 24 h) beneficial effects of DEX are independent of its' effect on brain edema.

CHAPTER SIX CONCLUSIONS AND RECOMMENDATIONS

As a result of these studies, significant advances have been made in the understanding of neurotransmitter alterations in peritumor brain and the effect of DEX on these changes. The hypothesis was that the presence of a brain tumor was associated with alterations in brain monoamine or GABA levels and that these changes were more severe in cats with abnormal neurologic signs of increased ICP. With the use of a suitable brain tumor model it was demonstrated that the presence of a tumor caused bilateral alterations in neurotransmitter amines, their metabolites, and GABA which were exacerbated in animals with abnormal neurologic signs.

Dexamethasone causes an improvement in the neurologic status of patients well before there is a reduction in brain edema; these experimental studies showed that this improvement may be at least partially due to changes in neurotransmitter metabolism. Treatment with DEX for 20 h (DEX 2) in animals without abnormal neurologic signs normalized monoamine levels in brain adjacent to the tumor; in cats with abnormal neurologic signs, treatment with DEX for 8 h (DEX 1) resulted in both an improvement in neurologic status and some improvements in neurotransmitter levels.

Malignant brain tumors are generally incurable. Thus, the ability to enhance these patients' quality of life by improving neurologic status is of considerable importance. These studies are first steps in determining whether commonly used drugs can alleviate the neurochemical changes seen in peritumor brain. One logical extension of these projects would be administering DEX for a longer time period in cats with abnormal neurologic signs to evaluate further changes in neurotransmitter levels. Another project could explore the link between surgical removal of the tumor and improvements in rCBF and rCGU (Mineura *et al.*, 1987); this brain tumor model would be excellent for defining and quantitating this phenomenon. It would also be important to determine whether surgical removal of the tumor +/- radiation therapy would alleviate depressed neurotransmitter metabolism.

New techniques such as *in vivo* brain dialysis would permit evaluation of sequential neurotransmitter changes in extracellular fluid and could be combined with assessment of neurologic status, EEG changes, and changes in direct cortical evoked responses during the period of drug administration. This should decrease measured variability because each animal could serve as its own control. The exact mechanism by which neurotransmitter amine metabolism is altered by DEX could be addressed using drugs which block synthesis or degradation. Known monoamine-altering drugs such as MAO inhibitors (e.g., phenelzine) or tricyclic antidepressants (e.g., imipramine) could be used; similar studies could be done using GABA-altering drugs. Comparison of drug effects in normal and tumor-bearing animals could lead to a better understanding of the many interactions of the tumor and associated edema on brain neurotransmitter metabolism. Perhaps a new role will be found for monoamine- or GABA-altering drugs in the treatment of neurosurgical disorders.

This brain tumor model could also be used for the study of other biochemical parameters. The levels of arachidonic acid, prostaglandins, and leukotrienes in the peritumor brain have not been extensively studied. Arachidonic acid and its metabolites play an important role in the pathophysiology of brain ischemia and brain injury (see chapter 1.3.8); they may be one cause of altered neurotransmitter levels in tumor-bearing brain. It would be interesting to see if neurochemical changes induced by DEX in peritumor brain are related to changes in levels of arachidonic acid or its metabolites. The nonglucocorticoid 21-aminosteroid U74OO6F can, like DEX, decrease or prevent vasogenic edema (Hall, personal communication) but can also inhibit CNS tissue lipid peroxidation via a α-tocopherol (vitamin E)-like membrane action and scavenge superoxide radicals (Braughler et al., 1987, 1988; Braughler, personal communication). Comparing the effects of DEX and U74006F on peritumor neurotransmitter levels may further help in elucidating the nature of depressed peritumor brain metabolism. If U74006F is equally effective, the lack of glucocorticoid activity of this compound suggests that it would be a safer agent then glucocorticoid steroids for the treatment of depressed peritumor brain metabolism.

It is generally thought that metabolic alterations in peritumor grey matter are a consequence of white matter edema and that the positive effect of DEX is due to its alleviation. In this study, however, treatment with DEX reversed the depletion of DA and its metabolites in peritumor grey matter without altering white matter edema. Improvement in neurologic status were also not related to a decrease in brain edema. This suggests a new concept in the treatment of patients with brain tumors -- one that would focus on reversing the metabolic alterations caused by the tumor, as well as decreasing the edema.

REFERENCES

Acz Z, Stark E (1978) Possible role of gamma-aminobutyric acid synthesis in the mechanism of dexamethasone feedback action. J Endocrinol 77:137-141

Aghajanan GK, Gallager DW (1975) Raphe origin of serotonergic nerves terminating in the cerebral ventricles. Brain Res 88:221-231

Agrawal HC, Davis JM, Himwich WA (1967) Water content of the developing kitten brain. J Neurochem 14:179-181

Alderman JL, Schellenberg MK (1974) γ-Aminobutyric acid (GABA) in rat brain: re-evaluation of sampling procedure and the postmortem increase. J Neurochem 22:937-940

Allison F, Adcock MH (1965) Failure of pretreatment with glucocorticoids to modify the phagocytic and bacterial capability of human leukocytes for encapsulated type I pneumococcus. J Bacteriol 89:1256-1261

Altenau LL, Kindt GW, McGauler JL (1977) Comparison of glucose metabolism in normal brain and edematous brain surrouding an experimental brain neoplasm. Acta Neurol Scand 56 [Suppl 64]:508-509

Amaral P, Sinnamon HM (1977) The locus coeruleus: neurobiology of a central noradrenergic nucleus. Prog Neurobiol 9: 147-196

Arcinue El, Mitchell RA, Sarnaik AP, McArthur B (1986) The metabolic course of Reye's syndrome: distinction between survivors and nonsurvivors. Neurology 36: 435-438

Ausman JI, Shapiro WR, Rull DP (1970) Studies on the chemotherapy of experimental brain tumors: development of an experimental model. Cancer Res 30:2394-2400

Baethmann A, Oethinger W, Chaussy L, Jesch F (1979) Corticosteroid effects on cerebral blood flow and metabolism; a potential mechanism in brain edema. Acta Neurol Scand 60 [Suppl 72]:370-371

Baethmann A, Van Harreveld A (1972) Physiological and biochemical findings in the central nervous system of adrenalectomized rats and mice. In Reulen HJ, Schürmann K (eds): Steroids and Brain Edema. Berlin: Springer-Verlag, pp 195-201

Baethmann A, Oettinger W, Moritake K, Chaussy L, Jesch F (1982) Metabolic effects of corticosteroids in central nervous tissue. In Hartmann A, Brock M (eds): Treatment of Cerebral Edema. Berlin: Springer-Verlag, pp 24-30

Banay-Schwartz M, Zanchin G, De Guzman T, Lajtha A (1979) The effect of corticosteroids on amino acid content of brain tissue preparations. Psychoneuroendocrinology 4:207-217

Baran H, Lassman H, Sperk G, Seitelberger F, Hornykiewicz O (1987) Effect of mannitol treatment on brain neurotransmitter markers in kainic acid-induced epilepsy. Neuroscience 21:679-684

Barber RP, Vaughn JE, Saito K, McLaughlin BJ, Roberts E (1978) GABAergic terminals are presynaptic to primary afferents in the substantia gelatinosa of the rat spinal cord. Brain Res 141:35-56

Bates D, Weinshilboum RM, Campbell RJ, Sundt Jr TM (1977) The effect of lesions in the locus coeruleus on the physiological responses of the cerebral blood vessels in cats. Brain Res 136:431-443

Baulieu EE (1978) Cell membranes, a target for steroid hormones. Mol Cell Endocrinol 12:247-254

Bayens-Simmonds J, Boisvert DPJ, Baker GB (1986) The effect of dexamethasone on brain neurotransmitter amines and their metabolites. Proc West Pharmacol Soc 29:3[illegible]-396

Bayens-Simmonds J, Boisvert DP, Baker GB (1987) Neurotransmitter amines, their metabolites, and amino acids in the 9L glioma and surrounding brain tissue in the cat. J Cereb Blood Flow Metab 7 [Suppl 1]:S475

Bayens-Simmonds J, Boisvert DPJ, Castro ME, Johnson ES (1988) A feline model for experimental studies of peritumor brain edema. J Neurooncol (in press)

Bazan NG, Tureo EBR (1980) Membrane lipids in pathogenesis of brain edema-phospholipids and arachidonic acid, the earliest membrane components changed at the onset of ischemia. In Cervós-Navarro J, Fersat R (eds): Advances in Neurology, Vol 28: Brain Edema. New York: Raven Press, pp 197-205

Beaney RP, Brooks DJ, Leenders KL, Thomas DGT, Jones T, Halnan KE (1985) Blood flow and oxygen utilisation in the contralateral cerebral cortex of patients with untreated intracranial tumours as studied by positron emission tomography with observations on the effect of decompressive surgery. J Neurol Neurosurg Psychiatry 48:310-319

Bell BA, Kean DM, MacDonald HL, Barnett HL, Douglas RHB, Smith MA, McGhee CNJ, Miller JD, Tocher JL, Best JJK (1987) Brain water measured by magnetic resonance imaging. Lancet 1:66-69

Benjamen RS, Wiernik PH, Bachar NR (1974) Adriamycin chemotherapy - efficacy, safety and pharmacological basis of intermittent single high dose schedule. Cancer 33:19-27

Bentivoglio M, Macchi G, Rossini P, Tempesta E (1978) Brain stem neurons projecting to the neocortex: a HRP study in the cat. Exp Brain Res 31:489-498

Bentue-Ferrer D, Reymann JM, Van Den Driessche J, Allain H, Bajot H (1985) Effect of triethyltin chloride on central aminergic neurotransmitters and their metabolites: relationship with pathology of aging. Exp Aging Res 11:137-141

Berndt SF (1982) Symptoms of cerebral edema. In Hartmann A, Brock M (eds): Treatment of Cerebral Edema. Berlin: Springer-Verlag, pp 36-42

Bigner DD (1977) Current status and future directions in brain tumor research. In Goldenson N, Appel S (eds): Scientific Approaches to Clinical Neurology. Philadelphia: Lea & Febiger, p 830

Bigner DD, Kvedar JP, Schaffer TC, Vick NA, Engel WK, Day ED (1972) Factors influencing the cell type of brain tumors induced in dogs by Schmidt-Ruppin rous sarcoma virus. J Neuropathol Exp Neurol 31:583-595

Bigner DD, Pegram CW, Vick NA, Copeland DD, Swenberg JA (1975) Characterization of the avian sarcoma virus-induced mammalian brain tumor model for immunologic and chemotherapeutic studies. In Környey St, Tariska St, Gosztonyi G (eds): Proceedings of VIIth International Congress of Neuropathology. Amsterdam: Excepta Medica, pp 445-451

Bigner SH, Bullard DE, Pegram CN, Wikstrand CJ, Bigner DD (1981) Relationship of in vitro morphologic and growth characteristics of established human glioma-derived cell lines to their tumorigenicity in athymic nude mice. J Neuropathol Exp Neurol 40:390-409

Bird ED, Iversen LL (1982) Human brain postmortem studies of neurotransmitters and related markers. In Lajtha A (ed): Handbook of Neurochemistry, Vol 2: Experimental Neurochemistry. New York: Plenum Press, pp 225-251

Bitoh S, Hasegawa H, Ohsuki H, Obashi J, Fujiwara M, Sakurai M (1984) Cerebral neoplasms initially presenting with massive intracerebral hemorrhage. Surg Neurol 22:57-62

Black KL, Hoff JT (1985) Leukotrienes increase blood-brain barrier permeability following intraparenchymal injections in rats. Ann Neurol 18:349-351

Black KL (1984) Leukotriene C_4 induces vasogenic cerebral edema in rats. Prostaglandins Leukotrienes Med 14:339-340

Black KL, Hoff JT, McGillicuddy JE, Gebarski SS (1986) Increased leukotriene C_4 and vasogenic edema surrounding brain tumors in humans. Ann Neurol 19:592-595

Blanc G, Hervé D, Simon H, Lisoprawski A, Glowinski J, Tassin JP (1980) Response to stress of mesocortico-frontal dopaminergic neurons in rats after long-term isolation. Nature 284:265-267

Blasberg RG, Shinohara M, Shapiro WR, Patlak CS, Pettigrew KD, Fenstermacher JD (1986) Apparent glucose utilization in Walker 256 metastatic brain tumors. J Neurooncol 4:5-16

Bodsch W, Rommel T, Ophoff BG, Menzel J (1987) Factors responsible for retention of fluid in human tumor edema and the effect of dexamethasone. J Neurosurg 67:250-257

Boismare F, Boquet J, Moore N, Chretien P, Salgaut C, Daoust M (1985) Hemodynamic, behavioral and biochemical disturbances induced by an experimental cranio-cervical injury (whiplash) in rats. J Auton Nerv Syst 13:137-147

Bohus B, DeKloet ER (1977) Behavioural effects of corticosterone related to putative glucocorticoid receptor properties in the rat brain. J Endocrinol 72:64P-65P

Bohus B, DeKloet ER (1981) Adrenal steroids and extinction behavior: Antagonism by progesterone, deoxycorticosterone and dexamethasone of a specific effect of corticosterone. Life Sci 28:433-440

Bonanno G, Raiteri M (1987) Coexistence of carriers for dopamine and GABA uptake on the same terminal in rat brain. Br J Pharmacol 91: 237-243

Bossi L (1986) Antiepileptic efficacy of GABA receptor agonists. In Nisticò G, Morselli PL, Lloyd KG, Fariello RG, Engel J (eds): Neurotransmitters, Seizures and Epilepsy III. New York: Raven Press, pp 473-484

Bothe H-W, Van den Kerckhoff W, Paschen W, Wallenfang T, Hossmann K-A (1982) Dissociation between blood flow and metabolic disturbances in edema associated with experimental abscess in cats. In Go KG, Baethmann A (eds): Recent Progress in the Study and Therapy of Brain Edema. New York: Plenum Press, pp 355-371

Bourke RS, Kimelberg HK, Nelson LR, Barron KD, Aven EL, Popp AJ, Waldman JB (1980) Biology of glial swelling in experimental brain edema. In Cervós-Navarro, Ferszt R (eds): Advances in Neurology, Vol. 28: Brain Edema. New York: Raven Press, pp 99-109

Bourke RS, Kimelberg HK, Dazé M, Church G (1983) Swelling and ion uptake in cat cerebrocortical slices: control by neurotransmitters and ion transport mechanisms. Neurochem Res 8:5-24

Brannan T, Weinberger J, Knott P, Taff I, Kaufmann H, Togasaki D, Nieves-Rosa J, Maker H (1987) Direct evidence of acute, massive striatal dopamine release in gerbils with unilateral strokes. Stroke 18: 108-110

Braughler JM (1985) Lipid peroxidation-induced inhibition of γ-aminobutyric acid uptake by rat brain synaptosomes: protection by glucocorticoids. J Neurochem 44: 1282-1288

Braughler JM, Chase RL, Neff GL, Yonkers PA, Dray JS, Hall ED, Sethy VH, Lahti RA (1988) A new 21-aminosteroid antioxidant lacking glucocorticoid acitivity stimulates ACTH secretion and blocks arachidonic acid release from mouse pituitary tumor (AtT-20) cells. J Pharmacol Exp Ther 244: 423-427

Braughler JM, Hall ED (1981) Acute enhancement of spinal cord synaptosomal (Na^+-K^+)-ATPase activity in cats following intravenous methylprednisolone. Brain Res 219:464-469

Braunschweiger PG, Ting HL, Schiffe LM (1983) Correlation between glucocorticoid receptor content and the antiproliferative effect of dexamethasone in experimental solid tumors. Cancer Res 43:4757-4761

Braughler JM, Pregenzer JF, Chase RL, Duncan LA, Jacobsen EJ, McCall JM (1987) Novel 21-aminosteroids as potent inhibitors of iron-dependent lipid peroxidation. J Biol Chem 262: 10438-10440

Brock M, Wiegand H, Zillig C, Zywietz C, Mock P, Dietz H (1976) The effect of dexamethasone on intracranial pressure in patients with supratentorial tumors (eds): In Pappius HM, Feindel W (eds): Dynamics of Brain Edema. Berlin, Springer-Verlag, pp 330-336

Brooks WH, Markesbery WR, Gupta GD, Roszman TL (1978) Relationship of lymphocytic invasian and survival of brain tumor patients. Ann Neurol 4:219-224

Brooks WH, Netsky MG, Normansell DE, Horwitz DA (1972) Depressed cell-mediated immunity in patients with primary intracranial tumors. J Exp Med 136:1631-1647

Brooksbank BWL, Atkinson DJ, Balacs R (1981) Biochemical development of the human brain: II. Some parameters of the GABA-ergic system. Dev Neurosci 4:188-200

Brostrom MA, Kon KC, Olson DR, Breckenridge B (1974) Adenosine 3',5'-monophosphate in glial tumor cells treated with glucocorticoids. Mol Pharmacol 10:711-720

Bruce JN, Criscuolo GR, Merrill MJ, Moquin RR, Blacklock JB, Oldfield EH (1987) Vascular permeability induced by protein product of malignant brain tumors: inhibition by dexamethasone. J Neurosurg 67:880-884

Bullard DE, Bigner DD (1983) Animal models and virus induction of tumors. In Walker MD (ed): Oncology of the Nervous System. Boston: M Nijhoff, pp 31-64

Buttinger C, Hartmann A, Von Kummer R, Menzel J (1982) The effect of high doses of dexamethasone on cerebral blood flow in patients with cerebral tumors. In Hartmann A, Brock M (eds): Treatment of Cerebral Edema. Berlin: Springer-Verlag, pp 132-138

Busto R, Harik SI, Yoshido S, Scheinberg P, Ginsberg MD (1985) Cerebral norepinephrine depletion enhances recovery after brain ischemia. Ann Neurol 18:329-336

Carpenter WT, Gruen PH (1982) Cortisol's effect on human mental functioning. J Clin Psychopharmacol 2:91-101

Carpenter WT Jr, Bunney WE Jr (1971) Adrenal cortical activity in depressive illness. Am J Psychiatry 128:31-40

Castro ME (1986) Experimental Brain Edema Studied with Proton NMR. PhD Thesis, University of Alberta. Edmonton, Alberta, Canada.

Chan PK, Fishman RA (1978) Brain edema: induction in cortical slices by polyunsaturated fatty acids. Science 201:358-360

Chan PK, Schmidley JW, Fishman RA, Longar SM (1984) Brain injury, edema, and vascular permeability changes induced by oxygen-derived free radicals. Neurology 34:315-320

Chan PK, Yurko M, Fishman RA (1982) Phospholipid degradation and cellular edema induced by free radicals in brain cortical slices. J Neurochem 38:525-531

Chang CC, Shinonaga M, Kuwabara T (1988) Effect of dexamethasone on neurotransmitter amines in a rat glioma model. In Long DM (ed): Advances in Neurology. New York: Raven Press, (in press)

Chapman AG, Riley K, Evans MC, Meldrum BS (1982) Acute effects of sodium valproate and γ-vinyl GABA on regional amino acid metabolism in the rat brain: incorporation of 2-[^{14}C] glucose into amino acids. Neurochem Res 7:1089-1105

Charnay DS, Redmond DE (1983) Neurobiological mechanisms in human anxiety. Neuropharmacol 22:1531-1536

Chelmicka-Schorr E, Arnason BG, Holshouser SJ (1980) C-6 glioma growth in rats: suppression with a beta-adrenergic agonist and a phosphodiesterase inhibitor. Ann Neurol 8:447-449

Chirigos MA, Humphreys SR (1966) Effect of alkylating agents on meningeal leukemia U210 arising in methotrexate-treated mice. Cancer Res 26:1673-1677

Chovanes GI, McAllister JP, Lamperti AA, Salotto AG, Truex RC (1988) Monoamine alterations during experimental hydrocephalus in neonatal rats. Neurosurgery 22:86-91

Clarke DE, Sampath SS (1975) Effects of hypophysectomy, bilateral adrenalectomy and hormone replacement therapy or organ monoamine oxidase activity. Experientia 31:1098-1100

Clasen RA, Cooke PM, Pandolfi S, Boyd D, Raimondi AJ (1962) Experimental cerebral edema produced by focal freezing 1. An anatomical study using vital dye techniques. J Neuropathol Exp Neurol 21:579-596

Cobb CA, Youmans JR (1982) Glial and neuronal tumours of the brain in adults. In Youmans JR (ed): Neurologic Surgery, Vol 5 (2nd ed). Philadelpia: WB Saunders Co, p 2809

Cochran ST, Higashida RT, Holburt E, Winter J, Norman A (1985) Development of rabbit brain tumor model for radiologic research. Invest Radiol 20:928-932

Cooper JR, Bloom FE, Roth RH (1986) The Biochemical Basis of Neuropharmacology (5th ed). New York, Oxford: Oxford University Press, pp 157, 225, 249-294

Copeland DD, Vogel FS, Bigner DD (1975) The induction of intracranial neoplasms by the inoculation of avian sarcoma virus in perinatal and adult rats. J Neuropathol Exp Neurol 34:340-358

Dafny N, Phillips MI, Taylor AN, Gilman S (1973) Dose effects of cortisol on single unit activities in hypothalamus, reticular formation and hippocampus of freely behaving rats correlated with plasma steroid levels. Brain Res 59:257-272.

Dahlgren N, Lindvall O, Sakabe T, Stenevi V, Siesjö B (1981) Cerebral blood flow and oxygen consumption in rat brain after lesions of noradrenergic locus coeruleus system. Brain Res 209:11-23

Daly J, Levitt M, Guroff G, Underfriend S (1968) Isotope studies on the mechanism of adrenal tyrosine hydroxylase. Arch Biochem Biophys 126:593-598

Dave VP, Dastur DK (1985) Correlation of fine structure with neurotransmitter levels in acute brain oedema in cats. Indian J Med Res 81:313-324

Davis JN, Carlsson A (1973) The effect of hypoxia on monoamine synthesis, levels, and metabolism in rat brain. J Neurochem 21:783-790

Davis JN, Carlsson A, MacMillan V, Siesjö BK (1973) Brain tryptophan hydroxylation: dependence on arterial oxygen tension. Science 182:72-74

DeKloet ER, Kovács GL, Szabó G, Telegdy G, Bohus B, Versteeg (1982) Decreased serotonin turnover in the dorsal hippocampus of rat brain shortly after adrenalectomy, selective normalization after corticosterone substitution. Brain Res 239:659-663

DeLaPaz RL, Patronas NJ, Brock RA (1983) Positron emission tomography study of suppression of grey matter glucose utilisation by brain tumours. AJNR 4:826-829

Demopolous HB, Flamm ES, Pietronigro DD, Seligman ML (1972) Molecular aspects of membrane structure in cerebral edema. In Reulen HJ, Schurmann K (eds): Steroids in Brain Edema. Berlin, New York: Springer-Verlag, pp 29-39.

DiChiro G, DeLaPaz R, Brooks RA, Sokoloff L, Kornblith PL, Smith BH, Patronas NJ, Kufta CV, Kessler RMM, Johnson GS, Manning RG, Wolf AP (1982) Glucose utilization of cerebral gliomas measured by [^{18}F] fluorodeoxyglucose and positron emission tomography. Neurology 32:1323-1329

Doczi T, Szerdahelyi P, Joc F (1984) 5-hydroxytryptamine injected intraventricularly fails to influence the brain water content. Neurosurgery 15:165-169

Dray A, Gonye TJ, Oakley NR, Tanner T (1976) Evidence for the existence of a raphe projection to the substantia nigra in rats. Brain Res 113:45-47

Duval D, Durant S, Homo-Delarche F (1983) Non-genomic effects of steroids. Interactions of steroid molecules with membrane structures and functions. Biochim Biophys Acta 737:409-422

Ebhardt G, Van den Kerckhoff W, Matsuoka Y, Hossmann K-A (1982) [Morphologische Befunde bei experimentell erzeugten gliomen in katzen]. Neurochirugia (Stuttg) 25:177-185

Edwards PM, Rousseau GG (1980) Effect of adrenalectomy and corticosteroids on three enzymes involved in amino acid metabolism in the brain of adult and newborn rats. J Steroid Biochem 13:567-569

Ehrenström F, Johnsson P (1985) A method for very rapid determination of catechols using ion-pairing reverse phase HPLC with electrochemical detection. Effects of L-dopa treatment on the catechol content in various brain structures. Life Sci 36:867-879

Ellison DW, Beal MF, Martin JB (1987) Amino acid neurotransmitters in postmortem human brain analyzed by high-perfomance liquid chromatography with electrochemical detection.
J Neurosci Methods 19:305-315

Erecinska M, Nelson D, Wilson DF, Silver IA (1984) Neurotransmitter amino acids in the CNS. I. Regional changes in amino acid levels in rat brain during ischemia and reperfusion. Brain Res 304:9-22

Fadda F, Argiolas A, Melis MR, Tissari AH, Onali PL, Gessa GL (1978) Stress-induced increases in 3,4-dihydroxyphenyacetic acid (DOPAC) levels in the cerebral cortex and nucleus accumbens: reversal by diazepam. Life Sci 23:2219-2224

Fagg GE (1985) L-Glutamate, excitatory amino acid receptors and brain function. Trends Neurosci 8:207-210

Fahn S, Côté LJ (1968) Regional distribution of γ-aminobutyric acid (GABA) in the brain of the rheseus monkey. J Neurochem 15:209-213

Faraj BA, Caplan DB, Newman SL, Ahmann PA, Camp VM, Kutner M (1984) Hypercatecholaminemia in Reye's Syndrome. Pediatrics 73:481-488

Faull KF, Bowersox SS, Qeller-De Amicis L, Maddaluno JF, Ciaranello RD, Dement WC (1988) Influence of freezer storage time on cerebral biogenic amine and metabolite concentrations and receptors ligand characteristics. Brain Res 450:225-230

Feeney DM, Baron JC (1986) Diaschisis. Stroke 17:817-830

Feldman S (1962) Electrophysiological alterations in adrenalectomy. Arch Neurol 7:460-470

Feldman S, Dafney N (1970) Effects of adrenocortical hormones on electrical activity of the brain. Progr Brain Res 32:90-100

Flower RJ, Blackwell GJ (1979) Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2-inhibitor which prevents prostaglandin generation. Nature 278:456-459

Folbergrova J, Ljunggren B, Norberg K, Siesjö, BK (1974) Influence of complete ischemia on glycolytic metabolites, citric acid cycle intermediates, and associated amino acids in the rat cerebral cortex. Brain Res 80:265-279

Fonnum F, Gottefeld Z, Grofova I (1978) Distribution of glutamate decarboxylase, choline acetyltransferase and aromatic amino acid decarboxylase in the basal ganglia of normal and operated rats. Evidence for striatopallidal, striatoentopeduncular and striatonigral gabaergic fibres. Brain Res 143:125-138

Forray C, Richelson (1985) Glucocorticoids potentiate the prostaglandin E_1-mediated cyclic AMP formation by a cultured murine neuroblastoma clone. J Neurochem 45:79-85

Foster SJ, Harden TK (1980) Dexamethasone increases β-adrenergic density in human astrocytoma cells. Biochem Pharmacol 27:2151-2153

Frattola L, Ferrarese C, Canal N, Gaini SM, Galluso R, Piotti R, Trabucchi M (1985) Characterization of the γ-aminobutyric acid receptor in human brain gliomas. Cancer Res 45:4495-4498

Galicich JH, French LA (1961) Use of dexamethasone in the treatment of cerebral edema resulting from brain tumors and brain surgery. Am Phys Dig Treat 12:169-174

Garcia-Bengochea F (1965) Cortisone and the cerebrospinal fluid of noncastrated cats. The Am Surg 31:123-125

Gardiner M, Nillson B, Rehncrona S, Siesjö BK (1981) Free fatty acids in rat brain in moderate and severe hypoxia. J Neurochem 36:1500-1505

Gardner WS, Miller WH (1980) Reversed-phase liquid chromatographic analysis of amino acids after reaction time with o-phthalaldehyde. Anal Chem 101:61-65

Gastaut JL, Michel B, Sabet L, Hassen S, Cerda M, Gastaut H (1979) Electroencephalography in brain edema (127 cases of brain tumor investigated by cranial computerized tomography). Electroencephalogr Clin Neurophysiol 46:239-255

Gilad G, Rabey J, Gilad UH (1987) Presynaptic effects of glucocorticoids on dopaminergic and cholinergic synaptosomes. Implications for rapid endocrine-neural interactions in stress. Life Sci 40:2401-2408

Glaser GH (1953) On the relationship between adrenal cortical activity and the convulsive state. Epilepsia 2:7-14

Glaser GH, Merrit HH (1952) Effects of corticotropin (ACTH) and cortisone on diseases of the nervous system. JAMA 148:898-904

Goeders NE, DeSouza EB, Kuhar MJ (1986) Benzodiazepine receptor- GABA ratios: regional differences in rat brain and modulation by adrenalectomy. Eur J Pharmacol 129:363-366

Goldman-Rakic PS, Brown RM (1981) Regional changes in monoamines in cerebral cortex and subcortical structures of aging rheseus monkeys. Neuroscience 6:177-187

Groothuis DR, Fischer JM, Vick NA, Bigner DD (1981) Comparative permeability of different glioma models to horseradish peroxidase. Cancer Treat Rep 65 [Suppl 2]:13-18

Gurcay O, Wilson C, Barker M (1971) Corticosteroid effect on transplantable rat glioma. Arch Neurol 24:266-269

Hagberg H, Lehmann A, Sandberg M, Nyström B, Jacobson I, Hamberger A (1985) Ischemia-induced shift of inhibitory and excitatory amino acids from intra- to extracellular components. J Cereb Blood Flow Metab 5:413-419

Haggstrom JE, Sjoquist B, Eckernas SA, Ingvast A, Gunne LM (1984) Discrete regional distributions of biochemical markers for the dopamine, noradrenaline, serotonin, GABA and acetylcholine systems in monkey brain (*Cebus apella*). Effects of stress. Acta Physiol Scand [Suppl 534]:1-27

Hall ED (1982) Acute effects of intravenous glucocorticoid on cat spinal motor neuron electrical properties. Brain Res 240:186-190

Hall ED, Baker T (1979) Acute effects of intravenous methylprednisolone sodium succinate on spinal reflexes. Exp Neurol 63:476-484

Hall ED, Baker T, Riker WF (1978) Glucocorticoid effects on spinal cord function. J Pharmacol Exp Ther 206:361-370

Hall ED, McGinley PA (1982) Effects of a single intravenous glucocorticoid dose on biogenic amine levels in cat lumbar spinal cord. J Neurochem 39:1787-1790

Hanbauer I, Guidofti A, Costa E (1975) Dexamethasone induces tyrosine hydroxylase in sympathetic ganglia but not in adrenal medulla. Brain Res 85:527-531

Hauptman M, Nelson D, Wilson DF, Erecinska M (1984) Neurotransmitter amino acids in the CNS. II. Some changes in amino acid levels in rat brain synaptosomes during and after in vitro anoxia and simulated ischemia. Brain Res 304:23-25

Haycock JW, Bennet WF, George RJ, Waymire JC (1982) Multiple site phosphorylation of tyrosine hydroxylase. Differential regulation in situ by 8-bromo-cAMP and acetylcholine. J Biol Chem 257:13699-13703

Hedley-Whyte ET, Hsu DW (1980) Effect of dexamethasone on normal blood-brain barrier. Fed Proc 39:774

Heistad DD (1981) Summary of symposium on cerebral blood flow: effect of nerves and neurotransmitters. J Cereb Blood Flow Metab 1:447-450

Hellstrom S, Commissiong J, Hanbauer I (1979) Modification of the dopamine and noradrenaline content in rat carotid body by carbohydrate-active steroids. Neuroscience 4:1157-1162

Hendry SHC, Jones EG (1981) Sizes and distributions of intrinsic neurons incorporating tritiated GABA in monkey sensory-motor cortex. J Neurosci 1:390-408

Henn FA, Hamberger A (1971) Glial cell function: Uptake of transmitter substances. Proc Nat Acad Sci USA 68:2868-2690

Herman JP, Guillonneau D, Dantzer R, Scatton B, Semerdjian-Rouquier L, LeMoal M (1982) Differential effects of inescapable footshocks and of stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat. Life Sci 30:2207-2214

Hikosaka O, Tanaka M, Sakamoto M, Iwamura Y (1985) Deficits in manipulative behaviours induced by local injections of musmicol into the first somatosensory cortex of the conscious monkey. Brain Res 325:375-380

Hirano A, Matsui T (1975) Vascular structures in brain tumors. Hum Pathol 6:611-621

Hong LS, Levine L (1976) Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. Proc Natl Acad Sci USA 73:1730-1734

Hossmann KA, Hürter T, Oschies U (1983) The effect of dexamethasone on serum protein extravascation and edema development in experimental brain tumors of cat. Acta Neuropathol (Berl) 60:223-231

Hossmann KA, Mies G, Paschen W, Szabo L, Dolan E, Wechsler W (1986) Regional metabolism of experimental brain tumors. Acta Neuropathol (Berl) 69:139-147

Hossmann KA, Niebuhr I, Tamura M (1982) Local cerebral blood flow and glucose consumption of rats with experimental gliomas.J Cereb Blood Flow Metab 2:25-32

Hossmann KA, Weschler W, Wilmes F (1979) Experimental peritumorous edema. Acta Neuropathol (Berl) 45:195-203

Ikarashi Y, Sasahara T, Maruyama Y (1985) Postmortem changes in catecholamines, indoleamines and their metabolites in rat brain regions: prevention with 10 kW microwave irradiation. J Neurochem 45:935-939

Ikeda Y, Mortsuura H, Nakazawa S (1984) Tissue concentrations of biogenic amines in experimental brain tumors. Nippon Ida Daigaku Zasshi 51:772-773

Ikeda Y, Nakasawa S (1981) Monoamine metabolites in ventricular and lumbar CSF of patients with brain tumors. Nippon Ida Daigaku Zasshi 48:704-7

Ikeda Y, Nakazawa S (1984) An analysis of the distributing pattern and tissue concentration of the biogenic amines in the well demarcated brain tumors and contrast enhancement by CT. Nippon Ida Daigaku Zasshi 51:132-133

Janisch W, Scheiber D (1977) Methods of induction of experimental CNS tumors. In Bigner DD, Swenberg JA (eds): Experimental Tumors of the Central Nervous System. Kalamazoo, Mich: The Upjohn Co, pp 16-41

Janisch W, Scheiber D (1977) Transplantation of CNS tumors. In Bigner DD, Swenburg JA (eds): Experimental Tumors of the Central Nervous System. Kalamazoo, Mich: The Upjohn Co, pp 64-69

Jellinger K, Riederer P, Kothbauer (1978) Changes in some putative neurotransmitters in human cerebral infarction. J Neural Transm [Suppl 14]:31-44

Johnston I, Gilay DL, Hendrick EB (1975) Experimental effects of steroids and steroid withdrawal on cerebrospinal fluid absorption. J Neurosurg 42:690-695

Kawamura H, Ito A, Oishi T, Yanaga T (1984) Influence of glucocorticoids on brain serotonin metabolism in rats. Endocrinol Jpn 31:595-603

Kawase T, Ohira T, Murakami H, Toya S, Shiga H (1985) Influence of peritumoral edema on rCBF and on cerebral function: analysis by xenon-enhanced CT and EEG topography. In Inaba Y, Klatzo I, Spatz M (eds): Brain Edema. Berlin: Springer-Verlag, pp 484-489

Keller HH, Bartolini G, Pletscher A (1973) Spontaneous and drug-induced changes of cerebral dopamine turnover during postnatal development of rats. Brain Res 64:371-378

Kim C, Campanelli C, Khanna JM (1983) Determination of picogram levels of brain catecholamines by a simplified liquid chromatographic electrochemical detection method. J Chromatogr 282:151-159

Kinney HC, Brody BA, Kloman AS, Gilles AS (1988) Sequence of central nervous system myelination in human infancy. II. Patterns of myelination in autopsied infants. J Neuropathol Exp Neurol 47:217-234

Klatzo I (1967) Neuropathological aspects of brain edema. Neuropathol Exp Neurol 26:1-14

Klatzo I, Chin E, Fujiwara K, Shulman K (1980) Resolution of vasogenic brain edema. In Cervós-Navarro J, Ferszt R (eds): Advances in Neurology, Vol 28: Brain Edema. New York: Raven Press, pp 359-374

Kontur P, Dawson R, Monjan A (1984) Manipulation of mobile phase parameters for HPLC separation of endogenous monoamines in rat brain tissue. J Neurosci Methods 11:5-18

Kostron H, Fischer J (1983) Regional, cellular and subcellular distribution of [^{3}H] dexamethasone in rat brain edema. Surg Neurol 20:48-54

Kramarcy NR, Delanoy RL, Dunn AJ (1984) Footshock treatment activates catecholamine synthesis in slices of mouse brain regions. Brain Res 290:311-319

Krieger DT, Silverberg AI, Rizzo F, Krieger HP (1968) Abolition of circadian periodicity of plasma 17-OHCS levels in the cat. Am J Physiol 215:959-967

Kuchiwachi H, Takada S, Misu N, Ishiguri H, Ikeda A, Kageyama N, Watari H (1985) Evaluation of steroid treatment of high-energy metabolism of ischemic brain edema in vivo by means of topical magnetic resonance (TMR-32) spectrometer. In Inada Y, Klatzo I, Spatz M (eds): Brain Edema. Berlin: Springer-Verlag, pp 187-193

Kumar S, Taylor G, Steward JK, Waghe MA, Morris-Jones D (1973) Cell-mediated immunity and blocking factors in patients with tumours of the central nervous system. Int J Cancer 12:194-205

Langfitt TW (1982) Increased intracranial pressure and the cerebral circulation. In Youmans JR (ed): Neurological Surgery, Vol 2 (2nd ed). Philadelphia: WB Saunders Co, pp 847, 876-880

Langlais PJ, Walsh FX, Steven TJJ, Bird ED (1983) Decreased catecholamine content caused by slicing of frozen human postmortem brain with an electric knife. Neurosci Lett 41:99-103

Leenders KL, Beaney RP, Brooks DJ, Lammerstsma AA, Heather JD, McKenzie CG (1985) Dexamethasone treatment of brain tumor patients: effects on regional cerebral blood flow, blood volume and oxygen utilization. Neurology 35:1610-1616

Lefauconnier JJ-M, Portemer C, Chatagner F (1976) Free amino acids and related substances in human glial tumours and in fetal brain: Comparison with normal adult brain. Brain Res 117:105-113

Leiguarda R, Sierra J, Pardal C, Zanbrano D (1985) Effect of large doses of methylprednisolone on supratentorial intracranial tumors. Eur Neurol 24:23-32

Levin VA, Freeman-Dove MA, Landahl HD (1975) Permeability characteristics of brain adjacent to tumours in rats. Arch Neurol 32:785-791

Lightman SL, Todd K, Everitt BJ (1984) Ascending noradrenergic projections from the brainstem: evidence for a major role in the regulation of blood pressure and vasopressin secretion. Exp Brain Res 55:145-151

Lindvall O, Björklund A, Moore RY, Stenevi U (1978) Mesencephalic dopamine neurons projecting to neocortex. Brain Res 81:325-331

Ling MHM, Perry PJ, Tsuang MT (1981) Side effects of corticosteroid therapy. Psychiatric aspects. Arch Gen Psychiatry 38:471-477

Lloyd KG, Davidson L, Price K (1977) Catecholamine and octopamine concentrations in brains of patients with Reye's syndrome. Neurology 27:985-988

London WT, Houff SA, Madden DL, Fucillo DA, Gravell M, Wallen WC, Palmer AE, Sever JL, Padgett BL, Walker DL, ZuRheim GM, Ohashi T (1978) Brain tumors in owl monkeys inoculated with a human polyomavirus (JC virus). Science 201:1246-1249

Long DM, Hartmann TF, French LA (1966) The response of human cerebral oedema to glucosteroid administration. An electron microscopic study. Neurology (Minn) 16:521-528

Long DM (1970) Capillary ultrastructure and the blood-brain barrier in human malignant brain tumors. J Neurosurg 32:127-144

Luthert PJ, Greenwood J, Lantos PL, Pratt OE (1986) The effect of dexamethasone on vascular permeability of experimental brain tumors. Acta Neuropathol (Berl) 69:288-294

Mansor LF, Holtkamp DE, Heming AE, Christian HH (1956) Effects of prednisone, prednisolone, and fludrocortisone acetate on electroshock seizure threshold. Fed Proc Fed Am Soc Exp Biol 15:454

Majewska M, Bisserbe J-C, Eskay RL (1985) Glucocorticoids are modulators of $GABA_A$ receptors in brain. Brain Res 339:178-182

Markowitsch HJ, Irle E (1981) Widespread cortical projections of the ventral tegmental area and of other brain stem structures in the cat. Exp Brain Res 41:233-246

Maruki C, Merkel W, Rausch WD, Spatz M (1982) Brain monoamines in cerebral ischemic edema, the effect of gamma-hydroxy-butyrate. In Go KG, Baethmann A (eds): Recent Progress in Study and Therapy of Brain Edema. New York: Plenum Press, pp 673-681

Maruo K, Veyama Y, Kubdahara Y, Hioki K, Saito M, Nomura T, Tamaoki N (1982) Human tumour xenografts in athymic rats and their age dependence. Br J Cancer 45:786-789

Matsumoto M, Kimura K, Fujisawa A, Matsuyama FU, Kunaga R, Yoneda S, Wada H, Abe H (1984) Differential effect of cerebral ischemia on monoamine content of discrete brain regions of the mongolian gerbil (*Meriones unguicutatus*). J Neurochem 42:647-651

Matsuoka S, Arakaki Y, Numaguchi K, Ueno S (1978) The effect of dexamethasone on electroencephalograms in patients with brain tumors. J Neurosurg 48:601-608

Matsuoka Y, Hossmann K-A (1981) Corticosteroid therapy of experimental tumour oedema. Neurosurg Rev 4:185-190

Maxwell RE, Long DM, French LA (1971) The effects of glucosteroids on experimental cold-induced brain edema. J Neurosurg 34:477-487

Mealey J, Chen TT, Schanz GP (1971) Effects of dexamethasone and methylprednisolone on cell cultures of human glioblastomas. J Neurosurg 34:324-334

Meaney MJ, Aitken DH (1985) [^{3}H] Dexamethasone receptors in rat frontal cortex. Brain Res 328:176-180

Menzies CB, Gunar M, Benhan PO (1980) Impaired thymus-derived lymphocyte function in patients with malignant brain tumor. Clin Neurol Neurosurg 82:157-168

Micco DJ, McEwen BS (1980) Glucocorticoids, the hippocampus, a behaviour: Interactive relation between task activation and steroid hormone binding activity. J Comp Psychol 4:624-633

Michelassi F, Landa L, Hill RD, Lowenstein E, Watkins WD, Patkau AJ, Zapol WM (1982) Leukotrienes D_4: a potent coronary artery vasoconstrictor associated with impaired ventricular contraction. Science 217;841-843

Mies G, Paschen W, Bodsch W, Hossmann KA (1983) Regional evaluation of blood flow and metabolism in experimental brain tumor of rats. In Ishii S, Nagai H, Brock M (eds): Intracranial Pressure V. Berlin: Springer-Verlag, pp 429-435

Miller JD, Leech P (1977) Effects of mannitol and steroid therapy on intracranial volume-pressure relationships in patients. J Neurosurg 42:274-281

Miller JD, Sakalas R, Ward JD, Adams WE, Vries JK, Becker DP (1977) Methylprednisolone treatment in patients with brain tumors. Neurosurgery 1:114-117

Miller JD, Stanek A, Langfitt TW (1971) Concepts of cerebral perfusion pressure and vascular compression during intracranial hypertension. Prog Brain Res 35:411-432

Mineura K, Yasuda T, Kowada M, Ogawa T, Shishido F, Vemura K (1987) Positron emission tomographic evaluation of radiochemotherapeutic effect on regional cerebral hemocirculation and metabolism in patients with gliomas. J Neurooncol 5:227-285

Mohanty S, Dey PK, Ray AK (1979) The role of serotonin in cerebral oedema. Indian J Med Res 69:1001-1007

Mokrasch LC (1971) Free amino acid content of normal and neoplastic rodent astroglia. Brain Res 25:672-676

Moore RY, Bloom FE (1978) Central catecholamine neuron systems: anatomy and physiology of the dopamine system. Ann Rev Neurosci 1:129-169

Morgan DF, Silberman AW, Bubbers JE, Rand RW, Storm FK, Morton DL (1982) An experimental brain tumor model in rabbits. J Surg Oncol 20:218-220

Moskowitz MA, Kiwak KJ, Hekimian K, Levine L (1984) Synthesis of compounds with properties of leukotrienes C_4 and D_4 in gerbil brains after ischemia and reperfusion. Science 224:886-888

Mrsulja BB, Mrsulja BJ, Spatz M, Ho U, Walker JT, Klatzo I (1976) Experimental cerebral ischemia in mongolian gerbils. Acta Neuropathol (Berl) 36:1-8

McEwen BS, DeKloet ER, Rostene W (1986) Adrenal steroid receptors and actions in the nervous system. Physiol Rev 66:1121-1188

McEwen BS, DeKloet ER, Wallach G (1976) Interactions in vivo and in vitro of corticoids and progesterone with cell nuclei and soluble macromolecules from rat brain regions and pituitary. Brain Res 105:129-136

McEwen BS, Krey LC, Luine VN (1978) Steroid hormone action in the neuroendocrine system: When is the genome involved? In Reichlin S, Baldessarini RJ, Martin JB (eds): The Hypothalamus. New York: Raven Press, pp 255-266

Naftchi NE, Demeny M, DeChresoto V, Tomasula JJ, Flamm ES, Campbell JB (1974) Biogenic amine concentrations in traumatized spinal cords of cats. Effect of drug therapy. J Neurosurg 40:52-57

Nagai T, McGeer PL, McGeer EG (1983) Distribution of GABA-T-intensive neurons in the rat forebrain and midbrain. Neurosci Lett 36:195-199

Nagao S, Momma F, Nishivra T, Murota T, Suga M (1986) Effect of methylprednisolone on cortical neural activity, blood flow and water content in air exposure cerebral edema. Exp Neurol 92:553-562

Nakagawa H, Groothuis DR, Owens ES, Fenstermacher JD, Patlak CS, Blasberg RG (1987) Dexamethasone effects on [^{125}I] albumin distribution in experimental RG-2 gliomas and adjacent brain. J Cereb Blood Flow Metab 7:687-701

Oppelt WW, Rall DP (1961) Production of convulsions in the dog with intrathecal corticosteroids. Neurology (Minneap) 11:925-7

Osterholm JL, Bell J, Meyer R, Pyenson J (1969) Experimental effects of free serotonin on the brain and its relation to brain injury. J Neurosurg 31:408-412

Osterholm JL (1981) Minireview: Noradrenergic mediation of traumatic spinal cord autodestruction. Life Sci 14:1363-1384

Otten V, Thoenen H (1977) Effect of glucocorticoids on nerve growth factor-mediated enzyme induction in organ cultures of rat sympathetic ganglia: enhanced response and reduced time requirement to initiate enzyme induction. J Neurochem 29:69-75

Pappius HM (1981) Local cerebral glucose utilization in thermally traumatized rat brain. Ann Neurol 9:484-491

Pappius HM (1982) Dexamethasone and local cerebral glucose utilization in freeze-traumatized brain. Ann Neurol 12:157-162

Pappius HM, Dadoun R (1986) Biogenic amines in injured brain (Abstr). Trans Am Soc Neurochem 17:298

Pappius HM, Dadoun R (1987) Effects of injury on the indoleamines in cerebral cortex. J Neurochem 49:321-325

Pappius HM, McCann WP (1969) Effects of steroids on cerebral edema in cats. Arch Neurol 20:207-216

Pappius HM, Wolfe LS (1983a) Involvement of serotonin and catecholamines in functional depression of traumatized brain. J Cereb Blood Flow Metab 3 [Suppl 1]:S226-S227

Pappius HM, Wolfe LS (1983b) Functional disturbances in brain following injury: search for underlying mechanisms. Neurochem Res 8:63-72

Pappius HM, Wolfe LS (1984) Effect of drugs on local cerebral glucose utilization in traumatized brain: Mechanisms of actions of steroids revisited. In Go KG, Baethmann A (eds): Recent Progress in the Study and Therapy of Brain Edema. New York: Plenum Press, pp 11-26

Paschen W, Djuric B, Mies G, Schmidt-Kastner R, Linn F (1987) Lactate and pH in the brain: Association and dissociation in different pathophysiological states. J Neurochem 48:154-159

Penn RD (1980a) Cerebral edema and neurological function in human beings. Neurosurgery 6:249-254

Penn RD (1980b) Cerebral edema and neurological function: CT, evoked responses, and clinical examination In Cervós-Navarro J, Ferszt R (eds): Advances in Neurology Vol 28: Brain Edema. New York: Raven Press, pp 383-394

Pericic D, Eng N, Walters JR (1978) Postmortem and aminooxyacetic acid-induced accumulation of GABA: Effect of gamma-butyrolactone and picrotoxin. J Neurochem 30:767-773

Perry TL, Berry K, Hansen S, Diamond S, Mok C (1971) Regional distribution of amino acids in human brain obtained by autopsy. J Neurochem 18:513-519

Perry TL, Hansen S, Berry K, Mok C, Lesk D (1971) Free amino acids and related compounds in biopsies of human brain. J Neurochem 18:521-528

Perry TL, Sanders HD, Hansen S, Lesk D, Kloster M, Gravlin L (1972) Free amino acids and related compounds of biopsied cat brain. J Neurochem 19:2651-2656

Phebus LA, Perry KW, Clemens JA, Fuller RW (1986) Brain anoxia releases striatal dopamine in rats. Life Sci 38:2447-2453

Phillipon J, Foncin JF, Grob R, Srour A, Poisson M, Pertuiset BF (1984) Cerebral edema associated with meningiomas: possible role of a secretory-excretory phenomenon. Neurosurgery 14:295-301

Phillips MI, Dafny N (1971) Effect of cortisol on unit activity in freely moving rats. Brain Res 25:651-655

Politi LE, Rodriguez de Turco EB, Bazan NG (1985) Dexamethasone effect of free fatty acid and diacylglycerol accumulation during experimentally induced vasogenic brain edema. Neurochem Pathol 3:249-270

Raichle ME, Hartman BK, Eichling JO, Sharpe LG (1975) Central noradrenergic regulation of cerebral blood flow and vascular permeability. Proc Natl Acad Sci USA 72:3726-3730

Raimondi AJ, Yashon D, Matsumoto S, Reyes CA (1967) Increased intracranial pressure without lateralizing signs. The midbrain syndrome. Neurochirugia (Stuttg) 10:197-209

Roma B, Spoerri O, Holzgraefe M, Mennel HD (1986) Current brain tumour models with particular consideration of the transplantation techniques. Acta Neurochir (Wien) 79:35-41

Rastogi RB, Singhal RL (1978) Adrenocorticoids control 5-hydroxytrytamine metabolism in rat brain. J Neural Transm 42:63-71

Reader TA, Masse P, De Champlain J (1979) The intracortical distribution of norepinephrine, dopamine and serotonin in the cerebral cortex of the cat. Brain Res 177:499-513

Reid AC, Teasdale GM, McCulloch J (1983) The effect of dexamethasone administration and withdrawal on water permeability across the blood-brain barrier. Ann Neurol 13:28-31

Reinhard JF, Bannon MJ, Roth RH (1982) Acceleration by stress of dopamine synthesis and metabolism in prefrontal cortex: antagonism by diazepam. Nauyn-Schmiedeberg's Arch Pharmacol 318:374-377

Reinoso-Suieréz F (1961) Topographischer Hiratlas der Katze. Darmstadt: Heraugegeben von E. Merck, pp AG, T5, T24, T15

Reivich M, Jones S, Ginsberg M, Slater R, Greenberg J (1977) Regional hemodynamic and metabolic alterations in focal cerebral ischemia: studies of diaschisis. Adv Exp Med Biol 94:617-622

Reulen HJ, Hadjidimos A, Schürmann K (1972) The effect of dexamethasone on water and electrolyte content and on rCBF in perifocal brain edema in man. In Reulen HJ, Schürmann K (eds): Steroids and Brain Edema. Berlin: Springer-Verlag, pp 239-252

Reulen HJ, Medzihradsky F, Ezenbach R, Marguth F, Brendal W (1969) Electrolytes, fluids and energy metabolism in human cerebral edema. Arch Neurol 21:517-524

Ribak CE (1978) Aspinous and sparsely-spinous stellate neurons in the visual cortex of cats containing glutamic acid decarboxylase. J Neurocytol 7:461-478

Ribak CE, Harris AB, Vaughn JE, Roberts E (1979) Inhibitory GABAergic nerve terminals decrease at sites of focal epilepsy. Science 205:211-214

Ridley A, Cavanagh JB (1969) Cellular reactions to heterologous, homologous, and autologous skin implanted into brain. J Pathol 99:193-203

Ridley A, Cavanagh JB (1971) Lymphocytic infiltration in gliomas: evidence of possible host resistance. Brain 94:117-124

Rieth KG, DiChiro G, London WT, Sever JL, Houff SA, Kornblith PL, McKeever PE, Buonomo C, Padgett BL, Walker DL (1980) Experimental glioma in primates: a computed tomography model. J Comput Assist Tomogr 4:285-290

Roberts DCS, Bloom FE (1981) Adrenal steroid induced changes in β-adrenergic receptor binding in rat hippocampus. Eur J Pharmacol 74:37-41

Rodriguez de Turco EB, Morelli de Liberti SA, Bazán NG (1983) Stimulation of free fatty acid and diacylglycerol production in cerebrum and cerebellum during bicuculline-induced status epilepticus. J Neurochem 40:252-259

Rosenblum ML, Deen DF, Hoshino T, Dougherty DA, Wilson CB (1980) Comparison of clonogenic cell assays after *in vivo* and *in vitro* treatment of 9L gliosarcoma. Br J Cancer 41 [Suppl 4]:307-308

Rothschild AJ, Langlais PJ, Schatzberg AF, Miller MM, Salomen MS, Lerbinger JE, Cole JO, Bird ED (1985) The effects of a single acute dose of dexamethasone on monoamine and metabolite levels in rat brain. Life Sci 36:2491-2501

Rubinstein LJ (1977) Correlation of animal brain tumor models with human neuro-oncology. *In* Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations. Natl Cancer Inst Monogr 46:43-49

Ruf K, Steiner FA (1967) Steroid-sensitive neurons in rat hypothalamus and midbrain: identification by microelectrophoresis. Science 156:667-669

Sadler SE, Maller JL (1982) Identification of a steroid receptor on the surface of xenopus oocytes by photoaffinity labelling. J Biol Chem 257:355-361

Saris SC, Bigner DD (1985) Experimental neuro-oncology. *In* Crockard A, Rayward R, Hoff JT (eds): Neurosurgery. Oxford: Blackwell Scientific Pub, pp 392-416

Saris SC, Bigner SH, Bigner DD (1984) Intracerebral transplantation of a human glioma cell line in immunosuppressed rats. J Neurosurg 60:582-588

Samuelsson B (1983) Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 220:568-575

Sato O (1967) The effect of dexamethasone on cerebral spinal fluid production rate in the dog. No To Shinkei 19:49-56

Schatzberg AF, Rothschild AJ, Stahl JB, Bond TC, Rosenbaum AH, Löfgren SB, MacLaughlin RA, Sullivan M, Cole JO (1983) The dexamethasone suppression test: identification of subtypes of depression. Am J Psychiat 140:88-91

Schatzberg AF, Rothschild AJ, Langlais PJ, Bird ED, Cole JO (1985) A corticosteroid/dopamine hypothesis for psychotic depression and related states. J Psychiat Res 19:57-64

Schaul N, Ball G, Gloor P, Pappius HM (1976) The EEG in cerebral edema. In Pappius HM, Feindel W (eds): Dynamics of Brain Edema. Berlin: Springer-Verlag, pp 144-149

Scheinberg LC, Edelman FL, Kevy WA (1964) Is the brain "an immunologically privileged site?" 1. Studies based on intracerebral tumor homotransplantation and isotransplantation to sensitized hosts. Arch Neurol 11:248-264

Schmidek HH, Nielson SL, Schiller AL, Messer J (1971) Morphological studies of rat brain tumors induced by N-nitrosomethylurea. J Neurosurg 34:335-340

Schmiedek P, Baethmann A, Sippel G, Oettinger W, Enzelbach R, Marguth F, Brendal W (1974) Energy state and glycolysis in human cerebral edema. The application of a new freeze-stop technique. J Neurosurg 40:351-364

Schmiedek P, Guggemos L, Baethmann A, Lanksch W, Kazner E, Picha B, Olteanu-Nerbe V, Enzenbach R, Brendel W, Marguth F (1976) Re-evaluation of short-term steroid therapy for perifocal brain edema. In Pappius HM, Feindel W (eds): Dynamics of Brain Edema. Berlin: Springer-Verlag, pp 344-350

Shapiro WR, Posner JB (1974) Corticosteroid hormones. Effects in an experimental brain tumor. Arch Neurol 30:217-221

Shenkin HA, Spitz EB, Grant FC, Kety SS (1948) The acute effects on the cerebral circulation of the reduction of increased intracranial pressure by means of intravenous glucose or ventricular drainage. J Neurosurg 5:466-70

Shibasaki T, Uki J, Kanoh T, Kawafuchi J-I (1979) Composition of free amino acids in brain tumours. Acta Neurol Scand 60:301-311

Shinohara M, Blasberg B, Patlak C (1979) Metastatic brain tumors: local cerebral glucose utilization. Neurology 29:545

Simon H, Scattan B, LeMoal M (1979) Dopaminergic A1O neurones are involved in cognitive function. Nature 286:150-151

Skipper HE, Schabel FM, Trader MW, Thompson JR (1966) Experimental evaluation of potential anticancer agents. VI. Anatomic distribution of leukemic cells and failure of chemotherapy. Cancer Res 26:1673-1677

Slouter RS, Conner JD (1977) Postmortem stability of norepinephrine, dopamine, and serotonin in rat brain. J Neurochem 28:1129-1131

Somogyi P, Cowey A, Halász N, Freund TF (1981) Vertical organization of neurones accumulating GABA in the visual cortex of rhesus monkey. Nature 294:761-763

Sovner R (1983) An amphetamine-like reaction to the dexamethasone suppression test in depressed patients. J Clin Psychopharmacol 3:236-238

Spokes EGS (1979) An analysis of factors influencing measurements of dopamine, noradrenaline, glutamic acid decarboxylase, and choline acetyltransferase in human autopsy brain material. Brain 102:333-346

Stastny F (1971) Hydrocortisone as a possible inductor of Na+-K+-ATPase in the chick embryo cerebral hemispheres. Brain Res 25:397-410

Sutton LE, Bruce DA, Welsh F (1980) The effects of cold-induced brain edema and white-matter ischemia on the somatosensory evoked response. J Neurosurg 53:180-184

Swartz SL, Dluhy RG (1978) Corticosteroids: Clinical pharmacology and therapeutic use. Drugs 16:238-255

Swenburg JA, Koestner A, Wechsler W, Denlinger RH (1972) Quantitative aspects of transplacental tumor induction with ethylnitrosourea in rats. Cancer Res 32:2656-2660

Sze PY, Neckers L, Towle AC, (1976) Glucocorticoids as a regulatory factor for brain tryptophan hydroxylase. J Neurochem 26:169-173

Szymas J, Hossmann KA (1984a) Immunofluoroscopic investigation of extravasation of serum proteins in human brain tumours and adjacent structures. Acta Neurochir (Wien) 71:229-241

Szymas J, Hossmann KA (1984b) Effect of dexamethasone on glial fibrillary acidic protein in peritumorous edema of cats: a morphometric study. Acta Neuropathol (Berlin) 62:309-315

Tabuchi K, Nishimoto A, Matsumoto K, Saton T, Nakasone S, Fujiwara T, Ogura H (1985) Establishment of a brain-tumor model in adult monkeys. J Neurosurg 63:912-916

Takeuchi J, Barnard O (1976) Perivascular lymphocytic cuffing in astrocytomas. Acta Neuropathol (Berl) 35:265-271

Takeuchi Y, Sano Y (1983) Immunohistochemical demonstration of serotonin fibres in the neocortex of the monkey (*Macaca fuscata*). Anat Embryol 166:155-168

Taki, W, Handa H, Isikawa M, Kobayashi M, Yamashita J, Yohekawa Y, Tanada S, Senda M, Yonekura Y, Torizuka K, Fujita R, Fukuyama H, Harada K, Fujimoto N, Kameyama M (1985) Local blood flow and oxygen metabolism in glioma and its surrounding brain. In Inada Y, Klatzo T, Spatz M (eds): Brain Edema. Berlin: Springer-Verlag, pp 267-272

Tallen HH, Moore S, Stein WH (1954) Studies on the free amino acids and related compounds in the tissues of the cat. J Biol Chem 211:927-939

Tanaka K, Marmarou A, Nishimura S (1983) Electrophysiological and regional cerebral blood flow changes associated with direct infusion edema. In Ishii S, Nagai H, Brock M (eds): Intracranial Pressure V. Berlin: Springer-Verlag, pp 413-418

Tang W, Sun GY (1982) Factors affecting the free fatty acids in rat brain cortex. Neurochem Int 4:269-273

Tank AW, Ham LN (1984) Regulation of tyrosine hydroxylase activity and mRNA for tyrosine hydroxylase by cyclic AMP and glucocorticoids in rat pheochromocytoma cell lines. Soc Neurosci Abstr 10:875

Tank AW, Weiner N (1982) Induction of tyrosine hydroxylase by glucocorticoids in mouse neuroblastoma cells. Enhancement of the induction by cyclic AMP. Mol Pharmacol 22:421-430

Thoenen H, Otten V (1978) Trans-synaptic enzyme induction: ionic requirements and modulatory role of glucocorticoids. In Usdin E, Weiner N, Youdim MBH (eds): Structure and Function of Monoamine Enzymes. New York: Marcel Dekker Inc, pp 439-464

Torbati ID, Harel D, Lavy S (1975) Excitability of the cortex in normal and adrenalectomized rats during repeated exposures to high oxygen pressure. Aviat Space Environ Med 46:241-243

Tork I, Turner S (1981) Histochemical evidence for a catecholaminergic (presumably dopaminergic) projection from the ventral mesencephalic tegmentum to visual cortex in the cat. Neurosci Lett 24:215-219

Tsubokawa T, Tsukiyama T, Ohta H, Kumakawa H (1985) Regional metabolism and circulation in peritumoral edema caused by meningioma and malignant glioma. In Inaba Y, Klatzo I, Spatz M (eds): Brain Edema. Berlin: Springer-Verlag, pp 194-199

Ushio Y, Chernik NL, Shapiro WR, Posner JB (1977) Metastatic tumor of the brain: development of an experimental model. Ann Neurol 2:20-29

Van Loon GR, Shum A, DeSouza ER (1981a) Brain serotonin turnover correlates inversely with plasma adrenocorticotropin during the triphasic response to adrenalectomy in rats. Endocrinology 108:2269-2276

Van Loon GR, Shum A, Sole MJ (1981b) Decreased brain serotonin turnover after short-term (two hour) adrenalectomy in rats: a comparison of four turnover methods. Endocrinology 108:1392-1402

Van Loon GR, Shum A, Sole MJ (1982) Decreased brain serotonin turnover in the dorsal hippocampus of rat brain shortly after adrenalectomy, selective normalization after corticosterone substitution. Brain Res 239:659-663

Veldhuis HD, DeKloet ER, VanZoest T, Bohus B (1982) Adrenalectomy reduces exploratory activity in the rat: A specific role of corticosterone. Hormon Behav 16:191-199

Vick N, Khandekar JD, Bigner DD (1977) Chemotherapy of brain tumors - the blood brain barrier is not a factor. Arch Neurol 34:523-526

Vincent SR, Hökfelt T, Skirball LR, Wu J-Y (1983) Hypothalamic γ-aminobutyric acid neurons project to the neocortex. Science 220:1309-1311

Von Metzlar A, Nitsch C (1981) [Effects of 3-methylcholanthrene and 3-methylcholanthrene plus piracetam on the γ-aminobutyric acid (GABA) content of several cerebral regions]. J Cancer Res Clin Oncol 101:339-343

Von Metzler A, Nitsch C (1985) Monoamine content in rat brain during carcinogenesis and the influence of the CNS drugs piracetam and imipramine. Naturwissenschaften 72:542-543

Waggener JD, Beggs JL (1976) Vasculature of neural neoplasms. In Thompson RA, Green JR (eds): Advances in Neurology, Vol 15: Neoplasia in the Central Nervous System. New York: Raven Press, pp 27-47

Walter WG (1936) The location of cerebral tumors by electroencephalography. Lancet 231:305-308

Walker MD, Alexander E, Hunt WE, McCarthy CS, Mahaley MS, Mealey J, Norrel HA, Owens G, Ransohoff J, Wilson CB, Gehan EA, Strike TA (1978) Evaluation of BCWV and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. J Neurosurg 49:333-343

Weizsaecker M, Deen DF, Rosenblum DF, Hoshino T, Gutin PH, Barker M (1981) The 9L rat brain tumor: description and application of an animal model. J Neurol 224:183-192

Weschler W, Ramadan MA, Gieseler A (1972) Isogenic transplantation of ethylnitrosourea-induced tumors of the central and peripheral nervous system in two different inbred rat strains. Naturwissenschaften 59:474

Weinberger J, Cohen G, Nieves-Rosa J (1983) Nerve terminal damage in cerebral ischemia: greater susceptibility of catecholamine nerve terminals relative to serotonin nerve terminals. Stroke 14:986-989

Weiss MH, Nulsen FE (1970) The effect of glucocorticoids on CSF flow in dogs. J Neurosurg 32:452-458

Welch KMA, Chabi E, Dodson RF, Wang T-PF, Nell J, Bergin B (1976) The role of biogenic amines in the progression of cerebral ischemia and edema: modification by p-chlorophenylalanine, methysergide, and pentoxyfilline. In Pappius HM, Feindel W (eds): Dynamics of Brain Edema. Berlin: Springer-Verlag, pp 193-202

Welsh FA, Rieder W (1978) Evaluation of in situ freezing of cat brain by NADH fluorescence. J Neurochem 31:299-309

Wiesel FA, Sedvall G (1974) Post-mortem changes of dopamine and homovanillic acid levels in rat striatum measured by mass fragmentography. Brain Res 65:547-549

Williams LR, Sandquist D, Black AC and Williams TH (1981) Glucocorticoids increase tyrosine hydroxylase activity in cultured murine neuroblastoma. J Neurochem 36:2057-2062

Wolfe LS, Pappius HM (1984) Arachidonic acid metabolites in cerebral ischemia and brain injury. In Bes A, Braquet P, Paoletti R, Siesjö BK (eds): Cerebral Ischemia. Amsterdam: Excepta Medica, pp 223-231

Wolleman M (1974) Biochemistry of Brain Tumors. London, Basingstoke: MacMillan, p 29.

Wood JD, Watson WJ, Ducker AJ (1968) The effect of hypoxia on brain γ-aminobutyric acid levels. J Neurochem 15:603-608

Woodbury DM (1954) Effects of hormones on brain excitability and electrolytes. In Pincus (ed): Recent Progress in Hormone Research, Vol10. New York: Academic Press, pp 65-107

Wurtman RJ, Axelrod J (1963) A sensitive and specific assay for the estimation of monoamine oxidase. Biochem Pharmacol 12:1439-1440

Yamada Y, Hayakawa T, Ushio Y, Kato A, Mogami H (1983) Tumor-induced brain edema and its treatment. A quantitative autoradiographic study. In Ishii S, Nagai H, Brock M (eds): Intracranial Pressure V. Berlin: Springer-Verlag, pp 436-441

Yamaura A, Saeki N, Makino H (1984) Presure waves of type A and B in patients with brain tumors with special reference to circadian rhythm and steroids. In Go KG, Baethmann A (eds): Recent Progress in the Study and Therapy of Brain Edema. New York: Plenum Press, pp 627-632

Yanase T, Nawata H, Higuchi K, Kato K, Ibayasha H (1984) Dexamethasone increases both catecholamines and methionine-enkephalin in cultured bovine adrenal chromaffin cells and human extramedullary pheochromocytoma cells. Life Sci 35:1869-1875 .

Yang MS, Lutz H, DeWitt DS, Becker DP, Hayes RL (1983) An improved method for in situ freezing of cat brain for metabolic studies. J Neurochem 41:1393-1397

Yan MH, Wright D, Nakagawa H, Blasberg R, Patlak C, Fenstermacher J (1985) Effects of dexamethasone on the blood-brain distribution of [^{125}I] albumin and [^{14}C] alpha-aminoisobutyric acid in vasogenic cerebral edema. In Inaba Y, Klatzo I, Spatz M (eds): Brain Edema. Berlin: Springer-Verlag, pp 638-645

Yeung J, Baker GB, Coutts RT (1986) Simple automated gas chromatography of amino acids and its application to brain tissue and urine. J Chromatogr Biomed Appl 378:293-304

Yoneda Y, Kanmori K, Ida S, Kuriyama K (1983) Stress-induced alterations in metabolism of γ-aminobutyric acid in rat brain. J Neurochem 40:350-356

Zanchin G, Maggioni F, Saladini M, Gabana MA, DaCol C, Andrioli G (1983) Amino acid transport in normal and tumoral human cerebral tissue. Acta Neurol (Napoli) 38:318-319

Zimmerman HM (1959) The nature of experimental gliomas. Clin Neurosurg 7:247-260

Zimmerman HM, Arnold H (1941) Experimental brain tumors I. Tumors produced by methylchloranthrene. Cancer Res 1:919-938

Zülch KJ (1965) Brain Tumors: their Biology and Pathology (2nd ed). New York: Springer-Verlag, p 26

Zülch KJ, Weschler W (1968) Pathology and classification of gliomas In Krayenbuhl H, Maspes PE, Sweet WH (eds): Progress in Neurological Surgery. Basel: Kargel, p 7